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(54) Title: INTEGRIN ALPHA SUBUNIT CYTOPLASMIC DOMAIN POLYPEPTIDES, ANTIBODIES AND METHODS

(57) Abstract

Diagnostic systems, methods, polypeptides and antibodies for detecting the presence of the cytoplasmic domain of the integrin  $\alpha_6\beta_1$  or  $\alpha_3\beta_1$  subunit in a body sample are disclosed.

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INTEGRIN ALPHA SUBUNIT CYTOPLASMIC  
DOMAIN POLYPEPTIDES, ANTIBODIES AND METHODS

Technical Field

5        The present invention relates to polypeptides that define the integrin  $\alpha_6$  and  $\alpha_3$  subunits, particularly the cytoplasmic domain of the  $\alpha_6$  and  $\alpha_3$  subunits. In addition, the invention describes 10      antibodies immunoreactive with the cytoplasmic domain of  $\alpha_6$  and  $\alpha_3$ , and methods for using the antibodies and polypeptides in assays for detecting  $\alpha_6$  and  $\alpha_3$  subunits in body samples.

Background

15      The integrin family of cell surface receptors serve cellular adhesion functions. The receptors form a link between the extracellular matrix and the cytoskeleton through their binding to various extracellular components. Each integrin receptor is a 20      heterodimer comprised of an  $\alpha$  and a  $\beta$  subunit. At least 11  $\alpha$  chains (Ruoslahti and Giancotti, 1989) and six  $\beta$  chains (Sheppard et al., 1990) have been 25      recognized in man. Each  $\alpha$  subunit tends to associate with only one type of  $\beta$  subunit, but there are several exceptions to this rule (Hemler et al., 1989; Cheresh et al., 1989; Holzmann et al., 1989; Freed et al., 1989).

30      The human heterodimer VLA-6 was identified using the monoclonal antibody GoH3, which is immunoreactive with the  $\alpha_6$  subunit expressed on the surface of mouse and human cells. Hemler et al. J. Biol. Chem., 263:7660-7665, (1988); and Sonnenberg et al. J. Biol. Chem., 262:10376-10383, (1987). The amino terminal sequence of the human VLA-6  $\alpha_6$  subunit was determined 35      from purified protein (Kajiji et al. EMBO J., 8:673-680, 1989) and was used to design degenerate

oligonucleotides for probing a cDNA library. The full length sequence of  $\alpha_6$  cDNA, and its predicted amino acid sequence, were elucidated subsequent to cDNA cloning. Tamura, et al., J. Cell Biol., 111:1593-1604 (1990). While Tamura et al., supra, also disclose multiple cDNA sequences encoding the VLA-6  $\beta_4$  subunit, there is provided no evidence that additional VLA-6  $\alpha_6$  subunits exist. European Patent Application Publication Number 279,669 (published July 24, 1988) describes human  $\alpha_6$  and  $\beta_4$  subunits of an integrin receptor and the complex they associate to form on pancreatic and other cancer cells. The publication does not describe or suggest that an isoform of the  $\alpha_6$  subunit exists.

The full length sequence of a hamster cDNA encoding the Gap b3 cell surface membrane glycoprotein was described by Tsuji et al., J. Biol. Chem., 265:7016-7021 (1990). Based on the predicted amino acid sequence and predicted overall structure, it was suggested that Gap b3 is the hamster homolog of the  $\alpha_3$  integrin subunit. The sequence of a cDNA encoding the partial sequence of chicken  $\alpha_3$  protein was disclosed in Hynes et al. J. Cell Biol., 109:409-420 (1989). The cytoplasmic regions of these clones do not share homology with the cytoplasmic region of  $\alpha_{3B}$  disclosed herein, and are therefore assumed to encode  $\alpha_{3A}$  subunit isoform. Furthermore, neither publication suggest the possibility of an  $\alpha_{3B}$  subunit.

The N-terminal amino acid sequence of human  $\alpha_3$  protein is provided in European Patent Application Publication Number 330,506 (published July 3, 1989). That publication provides no suggestion that an isoform of the  $\alpha_3$  protein, namely  $\alpha_{3B}$ , exists.

5       A new species of alpha ( $\alpha$ ) integrin subunit protein has been discovered, with representative members in both the  $\alpha_6$  and  $\alpha_3$ , class of integrins corresponding to the laminin receptor and the laminin, collagen and fibronectin receptors, respectively.

10      Specifically, it has been discovered that new  $\alpha_6$  species and  $\alpha_3$  species exist which differ from previously described  $\alpha_6$  and  $\alpha_3$  proteins in the cytoplasmic domain of the protein. Through a combination of cDNA sequencing studies and anti-synthetic peptide antibody immunoreactivity studies, it has been shown that the cytoplasmic domain of these new proteins, designated  $\alpha_{6B}$  and  $\alpha_{3B}$ , are related between human and mouse isolates.

15      Thus the present invention describes polypeptides comprising an amino acid residue sequence that includes the amino acid residue sequence defining an antigenic determinant in the cytoplasmic domain of the human or mouse  $\alpha_{6B}$  or  $\alpha_{3B}$  protein. Preferably, the polypeptide has a sequence corresponding to the whole cytoplasmic domain of either the human or mouse  $\alpha_{6B}$  or  $\alpha_{3B}$  protein. Alternatively, a polypeptide can correspond to all or substantially all of a native human or mouse  $\alpha_{6B}$  or  $\alpha_{3B}$  subunit in substantially isolated form.

20      The polypeptides or proteins are useful as immunogens for preparing polyclonal and monoclonal antibodies immunoreactive with the human or mouse  $\alpha_{6B}$  or  $\alpha_{3B}$  cytoplasmic domains, and as reagents for use in diagnostic assays for detecting the  $\alpha_{6B}$  or  $\alpha_{3B}$  proteins.

25      Thus, in a related embodiment the invention describes polyclonal and monoclonal antibodies having immunospecificities for antigenic determinants on the cytoplasmic domains of  $\alpha_{6B}$  and  $\alpha_{3B}$  proteins. These antibodies find use in in vitro and in situ

immunoassays for detecting  $\alpha_{6B}$  or  $\alpha_{3B}$  cytoplasmic domain antigens in body samples such as tissues or fluids.

Another aspect of the invention is the diagnostic methods and kits therefor, for detecting  $\alpha_{6B}$  or  $\alpha_{3B}$  cytoplasmic domain antigenic determinants using an antibody of this invention.

Other features and benefits of the invention will become apparent from the following detailed description and specific examples describing the invention, its principles and preferred embodiments.

#### Brief Description of the Drawings

In the drawings forming a portion of this disclosure:

Figure 1 illustrates immunoprecipitation of polypeptides from mouse cells using antibodies specific for the  $\alpha_6$  subunit. The differentiated (Diff.) ES1 and D3 cells are described in Example 2. Antibody GoH3 is a monoclonal antibody immunospecific for the extracellular domain of the  $\alpha_{6A}$  subunit. Antisera 6844 was raised in rabbit against a synthetic peptide specific for the cytoplasmic domain of human  $\alpha_{6A}$ . The immunoprecipitated labeled proteins were visualized by SDS-PAGE. Molecular weight, in kilodaltons, is noted on the side of the gel.

Figures 2 and 3 illustrate a sequential immunoprecipitation analysis of  $\alpha_6$  subunits in human JAR cell lysates as described in Example 2. NRS is normal rabbit preimmune sera, anti- $\alpha_6$  Mab is GoH3, anti- $\alpha_{6A}$  is sera 6844 and anti- $\alpha_{6B}$  is sera 382. The molecular weight of standard protein markers is shown on the right side of the gel and is expressed in kilodaltons (KDa). Figure 2 shows immunodepletion with NRS or with anti- $\alpha_6$  Mab, and Figure 3 shows immunodepletion with anti- $\alpha_{6A}$  or with anti- $\alpha_{6B}$ .

Figure 4 shows  $\alpha_{6A}$  and  $\alpha_{6B}$  PCR amplification products visualized on an ethidium stained gel. Single-stranded cDNA was generated from human PG, JAR and U937 cells and was amplified with a set of primers, 1156 and 1157, specific for the human  $\alpha_{6A}$  sequence as described in Example 3. The primers were also used to amplify the cloned human  $\alpha_{6A}$  cDNA sequence, which yielded an amplification product of about 540 bp. The amplification products from the tested cell lines were either 540 bp or 410 bp, or both.

Figure 5 compares the nucleotide sequences of the 540 bp and 410 bp amplification products described in Figure 4. The 540 bp product shown on the top line is designated  $\alpha_{6A}$ , and the 410 bp product shown on the bottom line is designated  $\alpha_{6B}$ . Vertical bars denote where the two sequences are homologous. Horizontal dots denote a 130 nucleotide (nt) deletion in the  $\alpha_{6B}$  sequence with respect to the  $\alpha_{6A}$  sequence. The 130 nt deletion is in the region that encodes the  $\alpha_{6A}$  cytoplasmic domain.

Figure 6 provides and compares the predicted amino acid sequence for the  $\alpha_6$  amplification products shown in Figure 5. The solid arrows show the location of the outer PCR primers; the broken arrows show the location of the nested inner PCR primers. The underlined sequence represent the putative transmembrane domain. The open boxed area is the  $\alpha_{6A}$  cytoplasmic domain; the shaded boxed area is the  $\alpha_{6B}$  cytoplasmic domain. The bracketed area represents the 130 nt sequence deleted from the  $\alpha_{6B}$  sequence.

Figure 7 depicts an ethidium bromide-stained gel of the PCR amplification products generated from (A) undifferentiated ES1 and B16 cells and (B) undifferentiated and differentiated ES1 cells as described in Example 3. The same priming

oligonucleotides were used to amplify cDNA from these cells.

Figure 8 provides and compares the nucleotide and predicted amino acid sequences for the mouse  $\alpha_6$  amplification products shown in Figure 7. The  $\alpha_{6B}$  sequence is on the top line; the  $\alpha_{6A}$  sequence is on the bottom line. Predicted amino acid residues are noted below the nucleotide sequence. The solid arrows show the location of the PCR primers. The boxed regions encompass the start of cytoplasmic domain for the  $\alpha_{6A}$  and  $\alpha_{6B}$  proteins, respectively.

Figure 9 illustrates the results of *in situ* immunostaining of diseased human kidney tissue. Panel A is stained with polyclonal antisera 6488 specific for the  $\alpha_{6A}$  cytoplasmic region. Panel B is stained with polyclonal antisera 382 specific for the  $\alpha_{6B}$  cytoplasmic region.

Brief Description of the Sequences in the Sequence Listing

The Sequence Listing is shown after the Examples and before the Claims.

SEQ ID NO 1 is the 1073 residue amino acid sequence of the human  $\alpha_{6A}$  protein. The putative transmembrane region is encompassed by amino acids 1012-1037. The mature protein is cleaved from the signal sequence between amino acids 23-24. Potential sites of N-linked glycosylation are at positions 223, 284, 370, 513, 731, 748, 891, 927 and 958. Putative cation binding domains are at positions 230-238, 324-332, 386-394 and 441-449. The cytoplasmic sequence GFFKR, which is conserved in virtually all of the integrin  $\alpha$  chains, begins at amino acid position 1040. The sequence encoded by the fragment of  $\alpha_{6A}$  cDNA amplified using primers 1156/1157 is encompassed by residues 927-1073.

SEQ ID NO 2 is the 5629 base nucleotide sequence of the human  $\alpha_{6A}$  cDNA. The initiating ATG is at nucleotide position 147. The mature coding sequence begins at nucleotide position 216 and ends at position 3365. The cytoplasmic sequence GFFKR is encoded by nucleotides 3264-3278. The 130 nucleotide sequence present in SEQ ID NO 2 but deleted from SEQ ID NO 4 is encompassed by nucleotides 3261-3390. The sequence of the  $\alpha_{6A}$  cDNA amplified using primers 1156/1157 is encompassed by nucleotides 2924-3455.

SEQ ID NO 3 is the 1091 residue amino acid sequence of the human  $\alpha_{6B}$  protein. The sequence of SEQ ID NO 3 is identical to SEQ ID NO 1 between amino acids 1 and 1044. The sequence encoded by the fragment of  $\alpha_{6B}$  cDNA amplified using primers 1156/1157 is encompassed by residue 927 through 1060.

SEQ ID NO 4 is the 5499 base nucleotide sequence of the human  $\alpha_{6B}$  cDNA. The sequence of SEQ ID NO 4 is identical to SEQ ID NO 2 between nucleotides 1 and 3260. Nucleotides 3261-5499 of SEQ ID NO 4 are identical to nucleotides 3391-5629 of SEQ ID NO 2. SEQ ID NO 4 has a 130 nucleotide deletion in relation to SEQ ID NO 2. The sequence of the  $\alpha_{6B}$  cDNA amplified using primers 1156/1157 is encompassed by nucleotides 2924-3325.

SEQ ID NO 5 is the 141 amino acid sequence predicted from the nucleic acid product which results from amplification of the mouse  $\alpha_{6B}$  cDNA with primers 1157/1156. The putative transmembrane domain begins at amino acid 88 and continues through amino acid 113. SEQ ID NO 5 is identical to SEQ ID NO 7 at amino acid position 1 through 120; the two sequences diverge at amino acid 121.

SEQ ID NO 6 is the 426 base nucleotide sequence corresponding to the mouse  $\alpha_{6B}$  amino acid sequence in SEQ ID NO 5. The putative transmembrane region is

encoded by nucleotides 262 through 337. SEQ ID NO 6 is identical to SEQ ID NO 8 except for 130 nucleotides present in SEQ ID NO 8 but deleted between nucleotides 342 and 343 of SEQ ID NO 6.

5 SEQ ID NO 7 is the 149 amino acid sequence predicted from the product which results from amplification of the mouse  $\alpha_{6A}$  cDNA with primers 1157/1156. SEQ ID NO 7 is identical to SEQ ID NO 5 at amino acid positions 1 through 120; the sequences 10 diverge at amino acid 121.

15 SEQ ID NO 8 is the 556 base nucleotide sequence corresponding to the mouse  $\alpha_{6A}$  amino acid sequence in SEQ ID NO 7, plus the first 109 nucleotides in the 3' noncoding region. SEQ ID NO 8 is identical to SEQ ID NO 6 except it has a 130 base insertion (nucleotides 342-472 of SEQ ID NO 8) between nucleotides 352 and 353 of SEQ ID NO 6.

20 SEQ ID NO 9 is the 153 amino acid sequence predicted from the product which results from amplification of the mouse  $\alpha_{3B}$  cDNA with primers 2032/2033. The cytoplasmic sequence CDFFK begins at amino acid position 108.

25 SEQ ID NO 10 is the 463 base nucleotide sequence corresponding to the mouse  $\alpha_{3B}$  amino acid sequence in SEQ ID NO 9. The cytoplasmic sequence CDFFK is encoded by nucleotides 324-338.

SEQ ID NO 11 is the outer 5' PCR primer 1157, corresponding to bp 2918-2937 of the  $\alpha_{6A}$  cDNA sequence of Sequence ID NO 2.

30 SEQ ID NO 12 is the outer 3' PCR primer 1156, corresponding to the complement of bp 3454-3473 of the  $\alpha_{6A}$  cDNA sequence of SEQ ID NO 2.

35 SEQ ID NO 13 is the inner 5' nested PCR primer 1681, corresponding to bp 2942-2960 of the  $\alpha_{6A}$  cDNA sequence of SEQ ID NO 2.

SEQ ID NO 14 is the inner 3' nested PCR primer 2002, corresponding to the complement of bp 3433-3452 of the  $\alpha_{6A}$  cDNA sequence of SEQ ID NO 2.

5 SEQ ID NO 15 is the 5' PCR primer 2032, corresponding to the hamster  $\alpha_{3A}$  cDNA sequence of Tsuji et al., J. Biol. Chem., 265:7016-7021 (1990).

10 SEQ ID NO 16 is the 3' PCR primer 2033, corresponding to the hamster  $\alpha_{3A}$  cDNA sequence of Tsuji et al., J. Biol. Chem., 265:7016-7021 (1990).

#### Detailed Description of the Invention

##### A. Definitions

15 Amino Acid Residue: An amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property is retained by the polypeptide. NH<sub>2</sub> refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard polypeptide nomenclature described in J. Biol. Chem., 243:3552-59 (1969) and adopted at 37 C.F.R. 1.822(b)(2), abbreviations for amino acid residues are shown in the following Table of Correspondence:

30

TABLE OF CORRESPONDENCE

| SYMBOL   |          | AMINO ACID    |
|----------|----------|---------------|
| 1-Letter | 3-Letter |               |
| Y        | Tyr      | tyrosine      |
| G        | Gly      | glycine       |
| 35 F     | Phe      | phenylalanine |
| M        | Met      | methionine    |

|    |   |     |               |
|----|---|-----|---------------|
|    | A | Ala | alanine       |
|    | S | Ser | serine        |
|    | I | Ile | isoleucine    |
|    | L | Leu | leucine       |
| 5  | T | Thr | threonine     |
|    | V | Val | valine        |
|    | P | Pro | proline       |
|    | K | Lys | lysine        |
|    | H | His | histidine     |
| 10 | Q | Gln | glutamine     |
|    | E | Glu | glutamic acid |
|    | W | Trp | tryptophan    |
|    | R | Arg | arginine      |
|    | D | Asp | aspartic acid |
| 15 | N | Asn | asparagine    |
|    | C | Cys | cysteine      |

It should be noted that all amino acid residue sequences are represented herein by formulae whose left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus. In addition, the phrase "amino acid residue" is broadly defined to include the amino acids listed in the Table of Correspondence and modified and unusual amino acids, such as those listed in 37 C.F.R. 1.822(b)(4), and incorporated herein by reference. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates either a peptide bond to a further sequence of one or more amino acid residues or a covalent bond to a carboxyl or hydroxyl end group.

Polypeptide and Peptide: Polypeptide and peptide are terms used interchangeably herein to designate a linear series of amino acid residues connected one to the other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues.

Protein: Protein is a term used herein to designate a linear series of greater than about 50 amino acid residues connected one to the other as in a polypeptide.

5           Synthetic peptide: refers to a chemically produced chain of amino acid residues linked together by peptide bonds that is free of naturally occurring proteins and fragments thereof.

10           Nucleotide: A monomeric unit of DNA or RNA consisting of a sugar moiety (pentose), a phosphate, and a nitrogenous heterocyclic base. The base is linked to the sugar moiety via the glycosidic carbon (1' carbon of the pentose) and that combination of base and sugar is a nucleoside. When the nucleoside 15 contains a phosphate group bonded to the 3' or 5' position of the pentose it is referred to as a nucleotide. A sequence of operatively linked nucleotides is typically referred to herein as a "base sequence" or "nucleotide sequence", and their grammatical equivalents, and is represented herein by 20 a formula whose left to right orientation is in the conventional direction of 5'-terminus to 3'-terminus.

25           Base Pair (bp): A partnership of adenine (A) with thymine (T), or of cytosine (C) with guanine (G) in a double stranded DNA molecule. In RNA, uracil (U) is substituted for thymine.

30           Nucleic Acid: A polymer of nucleotides, either single or double stranded.

35           Polynucleotide: a polymer of single or double stranded nucleotides. As used herein "polynucleotide" and its grammatical equivalents will include the full range of nucleic acids. A polynucleotide will typically refer to a nucleic acid molecule comprised of a linear strand of two or more deoxyribonucleotides and/or ribonucleotides. The exact size will depend on many factors, which in turn depends on the ultimate

conditions of use, as is well known in the art. The polynucleotides of the present invention include primers, probes, RNA/DNA segments, oligonucleotides or "oligos" (relatively short polynucleotides), genes, vectors, plasmids, and the like.

5 Gene: A nucleic acid whose nucleotide sequence codes for an RNA or polypeptide. A gene can be either RNA or DNA.

10 Duplex DNA: a double-stranded nucleic acid molecule comprising two strands of substantially complementary polynucleotides held together by one or more hydrogen bonds between each of the complementary bases present in a base pair of the duplex. Because the nucleotides that form a base pair can be either a 15 ribonucleotide base or a deoxyribonucleotide base, the phrase "duplex DNA" refers to either a DNA-DNA duplex comprising two DNA strands (ds DNA), or an RNA-DNA duplex comprising one DNA and one RNA strand.

20 Recombinant DNA (rDNA) molecule: a DNA molecule produced by operatively linking two DNA segments. Thus, a recombinant DNA molecule is a hybrid DNA molecule comprising at least two nucleotide sequences not normally found together in nature. rDNA's not 25 having a common biological origin, i.e., evolutionarily different, are said to be "heterologous".

30 Vector: a rDNA molecule capable of autonomous replication in a cell and to which a DNA segment, e.g., gene or polynucleotide, can be operatively linked so as to bring about replication of the attached segment. Vectors capable of directing the expression of genes encoding for one or more proteins are referred to herein as "expression vectors". Particularly important vectors allow cloning of cDNA 35 (complementary DNA) from mRNAs produced using reverse transcriptase.

B. Integrin Alpha Subunit Polypeptides

The present invention relates to a previously undescribed species of integrin alpha subunit that is derived by splicing of the messenger RNA in the tissue in which the integrin alpha subunit is expressed, such that the amino acid sequence of the alpha subunit polypeptide has a sequence as defined herein.

Splicing as a form of regulation of gene expression is one means by which a cell regulates the structural gene products expressed in that cell type. According to the structures defined herein, it is now known that the  $\alpha_6$  and  $\alpha_3$  integrin subunits can each be expressed in two alternate forms (isoforms), designated herein as an "A" form and a "B" form depending upon the spliced product, and are referred to as  $\alpha_{6A}$  or  $\alpha_{6B}$ , and as  $\alpha_{3A}$  or  $\alpha_{3B}$ .

The newly described  $\alpha_{6B}$  and  $\alpha_{3B}$  subunits contain a carboxyterminal amino acid residue sequence defining their cytoplasmic domain that is different from their  $\alpha_{6A}$  and  $\alpha_{3A}$  counterparts. These new species of  $\alpha_{6B}$  and  $\alpha_{3B}$  provide, based on their sequence differences, novel polypeptide reagents based on (1) the antigenic determinants present in their cytoplasmic domains and (2) the structural role the cytoplasmic domain of these proteins play in the function of the integrins of which they are members.

1.  $\alpha_{6B}$  Subunit Polypeptides

In one embodiment, the present invention contemplates a polypeptide based on the cytoplasmic domain of the  $\alpha_{6B}$  species of the integrin  $\alpha_6$  subunit. This polypeptide has an amino acid sequence that includes a sequence that corresponds, and preferably is identical to, the amino acid residue sequence of the cytoplasmic domain of the human or mouse  $\alpha_{6B}$ .

5           The cytoplasmic domain of human  $\alpha_{6B}$  includes an amino acid residue sequence shown in SEQ ID NO 3 from residue 1068 to residue 1091 and of mouse  $\alpha_{6B}$  has an amino acid residue sequence shown in SEQ ID NO 5 from residue 121 to residue 141.

10           Thus, in one embodiment, the present invention contemplates a polypeptide having an amino acid residue sequence that includes at least the sequence shown in SEQ ID NO 3 from residue 1068 to residue 1091 that defines the carboxy terminal portion of cytoplasmic domain of human  $\alpha_{6B}$ . Preferably a polypeptide has an amino acid residue sequence shown in SEQ ID NO 3 from residue 1068 to residue 1091, and more preferably has an amino acid residue sequence shown in SEQ ID NO 3 from residue 1045 to residue 15 1091. In a related embodiment the invention contemplates the whole human  $\alpha_{6B}$  protein, in a substantially isolated form, having a sequence shown in SEQ ID NO 3 from residue 1 to residue 1091.

20           By substantially isolated is meant that the protein is present in a composition as a major constituent, typically in amount greater than 10%, and preferably greater than 90%, of the total protein in the composition. Human  $\alpha_{6B}$  protein can be isolated by 25 a variety of biochemical and immunological means from the tissue sources and cells described herein that contain  $\alpha_{6B}$  subunit. Exemplary methods involve the use of a  $\alpha_{6B}$  cytoplasmic domain specific antibody, such as 382 described herein, alone or in combination 30 with the teachings of Kajiji et al., EMBO J., 8:673-680 (1989).

35           In a related embodiment, the present invention contemplates a polypeptide having an amino acid residue sequence that includes at least the sequence shown in SEQ ID NO 5 from residue 121 to residue 141 that defines a portion of the cytoplasmic domain of

mouse  $\alpha_{6B}$ . Preferably a polypeptide has an amino acid residue sequence shown in SEQ ID NO 5 from residue 121 to residue 141. Also contemplated is the whole mouse  $\alpha_{6B}$  protein in a substantially isolated form that 5 included a sequence shown in SEQ ID NO 5 from residue 1 to residue 141, with the degree of isolation being the same as above for human  $\alpha_{6B}$ . Purification 10 of mouse  $\alpha_{6B}$  can similarly be accomplished using the methods described above, and particularly using the murine cells described herein as a source of protein and an anti-peptide antibody prepared using mouse  $\alpha_{6B}$  cytoplasmic domain-derived polypeptides.

15 The native mouse  $\alpha_{6B}$  subunit polypeptide is a protein of about 125,000 daltons in molecular weight when analyzed by PAFE-SDS under reducing conditions as described in the Examples.

20 The native human  $\alpha_{6B}$  subunit polypeptide is a protein of about 125,000 daltons in molecular weight when analyzed by polyacrylamide-sodium dodecyl sulfate gel electrophoresis (PAGE-SDS) under reducing conditions as described in the Examples.

## 2. $\alpha_{3B}$ Subunit Polypeptides

25 In another embodiment, the present invention contemplates a polypeptide based on the cytoplasmic domain of the  $\alpha_{3B}$  species of the integrin  $\alpha_{3B}$  subunit. This polypeptide has an amino acid sequence that includes a sequence that corresponds, and preferably is identical to, the amino acid residue sequence of the cytoplasmic domain of the human or mouse  $\alpha_{3B}$ .

30 The cytoplasmic domain of mouse  $\alpha_{3B}$  has an amino acid residue sequence shown in SEQ ID NO 9 from residue 113 to residue 153.

35 Thus, in one embodiment, the present invention contemplates a polypeptide having an amino acid residue sequence that includes at least the sequence shown in SEQ ID NO 9 from residue 113 to residue 153

that defines a portion of the cytoplasmic domain of  $\alpha_{3B}$ . Preferably a polypeptide has an amino acid residue sequence shown in SEQ ID NO 9 from residue 113 to residue 153, and more preferably has an amino acid residue sequence shown in SEQ ID NO 9 from residue 1 to residue 153.

In a related embodiment, the invention contemplates the whole mouse  $\alpha_{3B}$  protein, in a substantially isolated form having a sequence that includes the sequence shown in SEQ ID NO 9 from residue 1 to residue 153. The degree of isolation for mouse  $\alpha_{3B}$  is the same as is for human  $\alpha_{6B}$  above, with methods for preparing the mouse  $\alpha_{3B}$  similarly based on immunoprecipitation or immunoaffinity isolation methods using an antibody specific for mouse  $\alpha_{3B}$  cytoplasmic domain as defined herein.

In preferred embodiments, a polypeptide of the present invention comprises about 20 to 1100 amino acid residues, and preferably comprises about 24 to 50 amino acid residues.

Preferably, a polypeptide of this invention is further characterized by its ability to immunologically mimic an epitope (antigenic determinant) expressed by the cytoplasmic domain of  $\alpha_{6B}$  or  $\alpha_{3B}$  as defined herein.

As used herein, the phrase "immunologically mimic" in its various grammatical forms refers to the ability of a polypeptide of this invention to immunoreact with an antibody of the present invention that recognizes an epitope on the cytoplasmic domain of  $\alpha_{6B}$  or  $\alpha_{3B}$  as defined herein.

It should be understood that a subject polypeptide need not be identical to the amino acid residue sequence of  $\alpha_{6B}$  or  $\alpha_{3B}$  so long as it includes a sequence that provides at least one epitope within the cytoplasmic domain of the  $\alpha_{6B}$  or  $\alpha_{3B}$  subunit and is

able to immunoreact with antibodies of the present invention.

A subject polypeptide includes any analog, fragment or chemical derivative of a polypeptide whose amino acid residue sequence is shown herein so long as the polypeptide is capable of immunologically mimicking a native epitope present in the cytoplasmic domain of  $\alpha_{6B}$  or  $\alpha_{3B}$ . Therefore, a polypeptide can be subject to various changes, substitutions, insertions, and deletions where such changes provide for certain advantages in its use. The term "analog" includes any polypeptide having an amino acid residue sequence substantially identical to a sequence specifically shown herein in which one or more residues have been conservatively substituted with a functionally similar residue and which displays the ability to mimic the cytoplasmic domain of  $\alpha_{6B}$  or  $\alpha_{3B}$  as described herein. Examples of conservative substitutions include the substitution of one non-polar (hydrophobic) residue such as isoleucine, valine, leucine or methionine for another, the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine, the substitution of one basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another.

The phrase "conservative substitution" also includes the use of a chemically derivatized residue in place of a non-derivatized residue provided that such polypeptide displays the requisite binding activity.

"Chemical derivative" refers to a subject polypeptide having one or more residues chemically derivatized by reaction of a functional side group. Such derivatized molecules include for example, those

molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butylloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine. Also included as chemical derivatives are those peptides which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids. For examples: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine. Polypeptides of the present invention also include any polypeptide having one or more additions and/or deletions or residues relative to the sequence of a polypeptide whose sequence is shown herein, so long as the requisite activity is maintained.

The term "fragment" refers to any subject polypeptide having an amino acid residue sequence shorter than that of a polypeptide whose amino acid residue sequence is shown herein.

When a polypeptide of the present invention has a sequence that is not identical to the sequence of the cytoplasmic domain of  $\alpha_{6B}$  or  $\alpha_{3B}$  because one or more conservative or non-conservative substitutions have been made, usually no more than about 30 number percent, more usually no more than 20 number percent, and preferably no more than 10 number percent of the amino acid residues are substituted, except that additional residues can be added at either terminus

for the purpose of providing a "linker" by which the polypeptides of this invention can be conveniently affixed to a label or solid matrix, or carrier, such that the linker residues do not form epitopes  
5 expressed by the cytoplasmic domain of  $\alpha_{6B}$  or  $\alpha_{3B}$  as defined herein. Labels, solid matrices and carriers that can be used with the polypeptides of this invention are described hereinbelow.

10 Amino acid residue linkers are usually at least one residue and can be 40 or more residues, more often 1 to 10 residues. Typical amino acid residues used for linking are tyrosine, cysteine, lysine, glutamic and aspartic acid, or the like. In addition, a subject polypeptide can differ, unless otherwise specified, from the natural sequence of an  $\alpha_{6B}$  or  $\alpha_{3B}$  cytoplasmic domain by the sequence being modified by terminal-NH<sub>2</sub> acylation, e.g., acetylation, or thioglycolic acid amidation, by terminal-carboxylamidation, e.g., with ammonia, methylamine, 15 and the like.  
20

When coupled to a carrier to form what is known in the art as a carrier-hapten conjugate, a polypeptide of the present invention is capable of inducing antibodies that immunoreact with the 25 cytoplasmic domain of either human or mouse  $\alpha_{6B}$  or mouse  $\alpha_{3B}$ . Where the immunogen is an  $\alpha_{3B}$ -derived polypeptide, the induced antibodies immunoreact with the cytoplasmic domain of either human or mouse  $\alpha_{3B}$ . This cross-reactivity between human and mouse 30 cytoplasmic domains is shown by the disclosures herein. In view of the well established principle of immunologic cross-reactivity, the present invention therefore contemplates antigenically related variants of the polypeptides of this invention. An 35 "antigenically related variant" is a subject polypeptide that is capable of inducing antibody

molecules that immunoreact with a subject polypeptide and with  $\alpha_{6B}$  or  $\alpha_{3B}$ .

Any peptide of the present invention may be used in the form of a pharmaceutically acceptable salt.

5 Suitable acids which are capable of forming salts with the peptides of the present invention include inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acetic acid, propionic acid, 10 glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalene sulfonic acid, sulfanilic acid or the like.

15 Suitable bases capable of forming salts with the peptides of the present invention include inorganic bases such as sodium hydroxide, ammonium hydroxide, potassium hydroxide and the like; and organic bases such as mono-, di- and tri-alkyl and aryl amines (e.g. triethylamine, diisopropyl amine, methyl amine, 20 dimethyl amine and the like) and optionally substituted ethanolamines (e.g. ethanolamine, diethanolamine and the like).

25 A polypeptide of the present invention, also referred to herein as a subject polypeptide, can be synthesized by any of the techniques that are known to those skilled in the polypeptide art, including recombinant DNA techniques. Synthetic chemistry techniques, such as a solid-phase Merrifield-type synthesis, are preferred for reasons of purity, 30 antigenic specificity, freedom from undesired side products, ease of production and the like. An excellent summary of the many techniques available can be found in J.M. Steward and J.D. Young, "Solid Phase Peptide Synthesis", W.H. Freeman Co., San Francisco, 35 1969; M. Bodanszky, et al., "Peptide Synthesis", John Wiley & Sons, Second Edition, 1976 and J. Meienhofer,

"Hormonal Proteins and Peptides", Vol. 2, p. 46,  
Academic Press (New York), 1983 for solid phase  
peptide synthesis, and E. Schroder and K. Kubke, "The  
Peptides", Vol. 1, Academic Press (New York), 1965 for  
5 classical solution synthesis, each of which is  
incorporated herein by reference. Appropriate  
protective groups usable in such synthesis are  
described in the above texts and in J.F.W. McOmie,  
10 "Protective Groups in Organic Chemistry", Plenum  
Press, New York, 1973, which is incorporated herein by  
reference.

In general, the solid-phase synthesis methods  
contemplated comprise the sequential addition of one  
or more amino acid residues or suitably protected  
15 amino acid residues to a growing peptide chain.  
Normally, either the amino or carboxyl group of the  
first amino acid residue is protected by a suitable,  
selectively removable protecting group. A different,  
selectively removable protecting group is utilized for  
20 amino acids containing a reactive side group such as  
lysine.

Using a solid phase synthesis as exemplary, the  
protected or derivatized amino acid is attached to an  
inert solid support through its unprotected carboxyl  
25 or amino group. The protecting group of the amino or  
carboxyl group is then selectively removed and the  
next amino acid in the sequence having the  
complimentary (amino or carboxyl) group suitably  
protected is admixed and reacted under conditions  
30 suitable for forming the amide linkage with the  
residue already attached to the solid support. The  
protecting group of the amino or carboxyl group is  
then removed from this newly added amino acid residue,  
and the next amino acid (suitably protected) is then  
35 added, and so forth. After all the desired amino  
acids have been linked in the proper sequence, any

remaining terminal and side group protecting groups (and solid support) are removed sequentially or concurrently, to afford the final polypeptide.

An  $\alpha_{6B}$  or  $\alpha_{3B}$ -derived polypeptide can be used, 5 inter alia, in the diagnostic methods and systems of the present invention to detect  $\alpha_{6B}$  or  $\alpha_{3B}$  present in a body sample, or can be used to prepare an inoculum as described herein for the preparation of antibodies that immunoreact with epitopes on the cytoplasmic 10 domain of either  $\alpha_{6B}$  or  $\alpha_{3B}$ .

#### C. DNA Segments

In living organisms, the amino acid residue sequence of a protein or polypeptide is directly related via the genetic code to the deoxyribonucleic acid (DNA) sequence of the structural gene that codes for the protein. Thus, a structural gene can be defined in terms of the amino acid residue sequence, 15 i.e., protein or polypeptide, for which it codes.

An important and well known feature of the 20 genetic code is its redundancy. That is, for most of the amino acids used to make proteins, more than one coding nucleotide triplet (codon) can code for or designate a particular amino acid residue. Therefore, a number of different nucleotide sequences may code 25 for a particular amino acid residue sequence. Such nucleotide sequences are considered functionally equivalent since they can result in the production of the same amino acid residue sequence in all organisms. Occasionally, a methylated variant of a purine or 30 pyrimidine may be incorporated into a given nucleotide sequence. However, such methylations do not affect the coding relationship in any way.

In one embodiment the present invention 35 contemplates an isolated DNA segment that comprises a nucleotide base sequence that encodes a polypeptide

that includes the amino acid residue sequence defining the cytoplasmic domain of  $\alpha_{6B}$  or  $\alpha_{3B}$  as defined herein.

5 A DNA segment therefor has a nucleotide sequence encoding the human or mouse  $\alpha_{6B}$  or mouse  $\alpha_{3B}$  proteins, or at least encoding the cytoplasmic domain of those proteins. The nucleotide sequences are generally shown in SEQ ID NO 4 for human  $\alpha_{6B}$ , NO 6 for mouse  $\alpha_{6B}$  and NO 10 for mouse  $\alpha_{3B}$ .

10 Preferred DNA segments include a nucleotide base sequence represented by the base sequence contained in SEQ ID NO 4 from base 3279 to base 3418 and defining a coding sequence that translates into the cytoplasmic domain of  $\alpha_{6B}$ . Particularly preferred is a nucleotide base sequence represented by the sequence contained in SEQ ID NO 4 from base 147 to base 3418 that defines the  $\alpha_{6B}$  integrin subunit. Corresponding nucleotide sequences for mouse  $\alpha_{6B}$  in SEQ ID NO 6 are also contemplated.

15

20 In another embodiment, preferred DNA segments include a nucleotide base sequence represented by the base sequence contained in SEQ ID NO 10 from base 339 to base 463 and defining a coding sequence that translates into the cytoplasmic domain of  $\alpha_{3B}$ . Particularly preferred is a nucleotide base sequence represented by the sequence contained in SEQ ID NO 10 from base 1 to base 463 that defines the carboxy 25 terminal portion of the  $\alpha_{3B}$  integrin subunit, including the cytoplasmic domain of  $\alpha_{3B}$ .

30 In preferred embodiments, the length of the nucleotide base sequence is no more than about 3,000 bases, preferably no more than about 1,000 bases.

35 A purified DNA segment of this invention is substantially free of other nucleic acids that do not contain the nucleotide base sequences specified herein for a DNA segment of this invention, whether the DNA segment is present in the form of a composition

containing the purified DNA segment, or as a solution suspension or particulate formulation. By substantially free is means that the DNA segment is present as at least 10% of the total nucleic acid present by weight, preferably greater than 50%, and more preferably greater than 90% of the total nucleic acid by weight.

5 In preferred embodiments, a DNA segment of the present invention is bound to a complementary DNA 10 segment, thereby forming a double stranded DNA segment. In addition, it should be noted that a double stranded DNA segment of this invention preferably has a single stranded cohesive tail at one or both of its termini.

15 A DNA segment of the present invention can easily be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci et al., J. Am. Chem. Soc., 103:3185 (1981). (The disclosures of the art cited herein are incorporated herein by 20 reference.) Of course, by chemically synthesizing the structural gene portion, any desired modifications can be made simply by substituting the appropriate bases 25 for those encoding a native amino acid residue.

25 In addition, a DNA segment can be prepared by first synthesizing oligonucleotides that correspond to portions of the DNA segment, which oligonucleotides are then assembled by hybridization and ligation into a complete DNA segment. Such methods are also well known in the art. See for example, Paterson et al., Cell, 48:441-452 (1987); and Lindley et al., Proc. Natl. Acad. Sci., 85:9199-9203 (1988), where a recombinant peptide, neutrophil-activated factor, was produced from the expression of a chemically synthesized gene in E. coli.

35 A DNA expression vector of the present invention is a recombinant DNA (rDNA) molecule adapted for

receiving and expressing translatable DNA sequences in the form of a fusion polypeptide of this invention. A DNA expression vector is characterized as being capable of expressing, in a compatible host, a structural gene product such as an  $\alpha_{63}$  or  $\alpha_{38}$  polypeptide of the present invention.

As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting between different genetic environments another nucleic acid to which it has been operatively linked. Preferred vectors are those capable of autonomous replication and expression of structural gene products present in the DNA segments to which they are operatively linked.

As used herein, the term "operatively linked", in reference to DNA segments, describes that the nucleotide sequence is joined to the vector so that the sequence is under the transcriptional and/or translation control of the expression vector and can be expressed in a suitable host cell.

The choice of vector to which a structural gene is operatively linked depends directly, as is well known in the art, on the functional properties desired, e.g., vector replication and protein expression, and the host cell to be transformed, these being limitations inherent in the art of constructing recombinant DNA molecules.

In preferred embodiments, the vector utilized includes a prokaryotic replicon i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extra chromosomally in a prokaryotic host cell, such as a bacterial host cell, transformed therewith. Such replicons are well known in the art. In addition, those embodiments that include a prokaryotic replicon also include a gene whose expression confers a selective advantage, such as drug resistance, to a

bacterial host transformed therewith. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline. Vectors typically also contain convenient restriction sites for insertion of translatable DNA sequences. Exemplary vectors are the plasmids pUC8, pUC9, pBR322, and pBR329 available from BioRad Laboratories, (Richmond, CA) and pPL and pKK223 available from Pharmacia, (Piscataway, NJ). Also contemplated are vectors for expressing a DNA segment of this invention in a yeast or mammalian host cell.

DNA expression control sequences include both 5' and 3' elements, as is well known, to form a cistron able to express a structural gene product. The 5' control sequences define a promoter for initiating transcription and a ribosome binding site operatively linked at the 5' terminus of the upstream translatable DNA sequence.

To achieve high levels of gene expression in *E. coli*, it is necessary to use not only strong promoters to generate large quantities of mRNA, but also ribosome binding sites to ensure that the mRNA is efficiently translated. In *E. coli*, the ribosome binding site includes an initiation codon (AUG) and a sequence 3-9 nucleotides long located 3-11 nucleotides upstream from the initiation codon [Shine et al., *Nature*, 254:34 (1975)]. The sequence, AGGAGGU, which is called the Shine-Dalgarno (SD) sequence, is complementary to the 3' end of *E. coli* 16S mRNA. Binding of the ribosome to mRNA and the sequence at the 3' end of the mRNA can be affected by several factors:

- (i) The degree of complementarity between the SD sequence and 3' end of the 16S tRNA.
- 35 (ii) The spacing and possibly the DNA sequence lying between the SD sequence and the AUG [Roberts et

al., Proc. Natl. Acad. Sci. USA, 76:760 (1979a);  
Roberts et al., Proc. Natl. Acad. Sci. USA, 76:5596  
(1979b); Guarente et al., Science, 209:1428 (1980);  
and Guarente et al., Cell, 20:543 (1980).]

5 Optimization is achieved by measuring the level of  
expression of genes in plasmids in which this spacing  
is systematically altered. Comparison of different  
mRNAs shows that there are statistically preferred  
sequences from positions -20 to +13 (where the A of  
10 the AUG is position 0) [Gold et al., Annu. Rev.  
Microbiol., 35:365 (1981)]. Leader sequences have  
been shown to influence translation dramatically  
(Roberts et al., 1979 a, b supra).

15 (iii) The nucleotide sequence following the AUG,  
which affects ribosome binding [Taniguchi et al., J.  
Mol. Biol., 118:533 (1978)].

#### D. Antibodies and Monoclonal Antibodies

20 The term "antibody" in its various grammatical  
forms is used herein as a collective noun that refers  
to a population of immunoglobulin molecules and/or  
immunologically active portions of immunoglobulin  
molecules, i.e., molecules that contain an antibody  
combining site or paratope.

25 An "antibody combining site" is that structural  
portion of an antibody molecule comprised of heavy and  
light chain variable and hypervariable regions that  
specifically binds (immunoreacts with) antigen. The  
term immunoreact in its various forms means specific  
30 binding between an antigenic determinant-containing  
molecule and a molecule containing an antibody  
combining site such as a whole antibody molecule or a  
portion thereof.

35 The phrase "antibody molecule" in its various  
grammatical forms as used herein contemplates both an

intact immunoglobulin molecule and an immunologically active portion of an immunoglobulin molecule.

Exemplary antibody molecules for use in the diagnostic methods and systems of the present invention are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contain the paratope, including those portions known in the art as Fab, Fab', F(ab')<sub>2</sub> and F(v).

Fab and F(ab')<sub>2</sub> portions of antibodies are prepared by the proteolytic reaction of papain and pepsin, respectively, on substantially intact antibodies by methods that are well known. See for example, U.S. Patent No. 4,342,566 to Theofilopolous and Dixon. Fab' antibody portions are also well known and are produced from F(ab')<sub>2</sub> portions followed by reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and followed by alkylation of the resulting protein mercaptan with a reagent such as iodoacetamide. An antibody containing intact antibody molecules are preferred, and are utilized as illustrative herein.

An antibody of the present invention in one embodiment is an anti-cytoplasmic  $\alpha_{6B}$  domain antibody characterized as being capable of immunoreacting with 1) human  $\alpha_{6B}$ , and 2) a polypeptide having a sequence shown in SEQ ID NO 3 from residue 1045 to residue 1091.

In another embodiment an antibody of this invention is an anti-cytoplasmic  $\alpha_{6B}$  domain antibody characterized as being capable of immunoreacting with 1) human  $\alpha_{6B}$ , and 2) a polypeptide having a sequence shown in SEQ ID NO 3 from residue 1068-1091.

In another embodiment an antibody of this invention is an anti-cytoplasmic  $\alpha_{6B}$  domain antibody

characterized as being capable of immunoreacting with 1) mouse  $\alpha_{6B}$  and 2) a polypeptide having a sequence shown in SEQ ID NO 5 from residue 121 to residue 141.

5 In another embodiment, an anti-cytoplasmic  $\alpha_{3B}$  domain antibody is contemplated that is characterized as being capable of immunoreacting with 1) mouse  $\alpha_{3B}$ , and 2) the polypeptide having a sequence shown in SEQ ID NO 9 from residue 113 to residue 153.

10 Antibody immunoreactivity with antigens containing a cytoplasmic domain as defined above can be measured by a variety of immunological assays known in the art. Exemplary immunoreaction of a subject antibody with  $\alpha_{6B}$  or  $\alpha_{3B}$  polypeptides is described in Examples 2 and 4 .

15 For example, immunoreaction with whole protein can be measured by the immunoprecipitation procedures described in Example 2. Immunoreaction of antibodies with polypeptides can be conveniently measured using ELISA as described in U.S. Patents No. 3,643,090; No. 20 3,850,752; or No. 4,016,043, which are incorporated herein by reference, using the polypeptide in the solid phase, as is well known.

25 An antibody of the present invention is typically produced by immunizing a mammal with an inoculum containing a polypeptide of this invention and thereby induce in the mammal antibody molecules having immunospecificity for the polypeptide. Exemplary immunization procedures for preparing an antibody of this invention are described in Example 2. The 30 antibody molecules are then collected from the mammal and isolated to the extent desired by well known techniques such as, for example, by using DEAE Sephadex to obtain the IgG fraction.

35 To enhance the specificity of the antibody, the antibodies may be purified by immunoaffinity chromatography using solid phase-affixed immunizing

5 polypeptide. The antibody is contacted with the solid phase-affixed immunizing polypeptide for a period of time sufficient for the polypeptide to immunoreact with the antibody molecules to form a solid phase-affixed immunocomplex. The bound antibodies are separated from the complex by standard techniques.

10 The antibody so produced can be used, *inter alia*, in the diagnostic methods and systems of the present invention to detect  $\alpha_{6\beta}$  or  $\alpha_{3\beta}$  subunits present in a body sample. See, for example, the methods described in Examples 2 and 4.

15 The word "inoculum" in its various grammatical forms is used herein to describe a composition containing a polypeptide of this invention as an active ingredient used for the preparation of antibodies against the cytoplasmic domain of an  $\alpha_{6\beta}$  or  $\alpha_{3\beta}$  polypeptide. When a polypeptide is used in an inoculum to induce antibodies it is to be understood that the polypeptide can be used in various 20 embodiments, e.g., alone or linked to a carrier as a conjugate, or as a polypeptide polymer. However, for ease of expression and in context of a polypeptide inoculum, the various embodiments of the polypeptides of this invention are collectively referred to herein 25 by the term "polypeptide", and its various grammatical forms.

30 For a polypeptide that contains fewer than about 35 amino acid residues, it is preferable to use the peptide bound to a carrier for the purpose of inducing the production of antibodies.

35 One or more additional amino acid residues can be added to the amino- or carboxy-termini of the polypeptide to assist in binding the polypeptide to a carrier. Cysteine residues added at the amino- or carboxy-termini of the polypeptide have been found to be particularly useful for forming conjugates via

disulfide bonds. However, other methods well known in the art for preparing conjugates can also be used. Exemplary additional linking procedures include the use of Michael addition reaction products, di-aldehydes such as glutaraldehyde, Klipstein, et al., J. Infect. Dis., 147:318-326 (1983) and the like, or the use of carbodiimide technology as in the use of a water-soluble carbodiimide to form amide links to the carrier. For a review of protein conjugation or coupling through activated functional groups, see Aurameas, et al., Scand. J. Immunol., 1:7-23 (1978).

Useful carriers are well known in the art, and are generally proteins themselves. Exemplary of such carriers are keyhole limpet hemocyanin (KLH), edestin, thyroglobulin, albumins such as bovine serum albumin (BSA) or human serum albumin (HSA), red blood cells such as sheep erythrocytes (SRBC), tetanus toxoid, cholera toxoid as well as polyamino acids such as poly (D-lysine: D-glutamic acid), and the like.

The choice of carrier is more dependent upon the ultimate use of the inoculum and is based upon criteria not particularly involved in the present invention. For example, a carrier that does not generate an untoward reaction in the particular animal to be inoculated should be selected.

The present inoculum contains an effective, immunogenic amount of a polypeptide of this invention, typically as a conjugate linked to a carrier. The effective amount of polypeptide per unit dose sufficient to induce an immune response to the immunizing polypeptide depends, among other things, on the species of animal inoculated, the body weight of the animal and the chosen inoculation regimen as is well known in the art. Inocula typically contain polypeptide concentrations of about 10 micrograms to about 500 milligrams per inoculation (dose),

preferably about 50 micrograms to about 50 milligrams per dose.

The term "unit dose" as it pertains to the inocula refers to physically discrete units suitable as unitary dosages for animals, each unit containing a predetermined quantity of active material calculated to produce the desired immunogenic effect in association with the required diluent; i.e., carrier, or vehicle. The specifications for the novel unit dose of an inoculum of this invention are dictated by and are directly dependent on (a) the unique characteristics of the active material and the particular immunologic effect to be achieved, and (b) the limitations inherent in the art of compounding such active material for immunologic use in animals, as disclosed in detail herein, these being features of the present invention.

Inocula are typically prepared from the dried solid polypeptide-conjugate by dispersing the polypeptide-conjugate in a physiologically tolerable (acceptable) diluent such as water, saline or phosphate-buffered saline to form an aqueous composition.

Inocula can also include an adjuvant as part of the diluent. Adjuvants such as complete Freund's adjuvant (CFA), incomplete Freund's adjuvant (IFA) and alum are materials well known in the art, and are available commercially from several sources.

The techniques of polypeptide conjugation or coupling through activated functional groups presently known in the art are particularly applicable. See, for example, Aurameas, et al., Scand. J. Immunol., Vol. 8, Suppl. 7:7-23 (1978) and U.S. Patent No. 4,493,795, No. 3,791,932 and No. 3,839,153. In addition, a site directed coupling reaction can be carried out so that any loss of activity due to

polypeptide orientation after coupling can be minimized. See, for example, Rodwell et al., Biotech., 3:889-894 (1985), and U.S. Patent No. 4,671,958.

5 One or more additional amino acid residues may be added to the amino- or carboxy-termini of the polypeptide to assist in binding the polypeptide to form a conjugate. Cysteine residues, usually added at the carboxy-terminus of the polypeptide, have been  
10 found to be particularly useful for forming conjugates via disulfide bonds, but other methods well-known in the art for preparing conjugates may be used.

15 A preferred antibody of this invention is a monoclonal antibody.

20 The phrase "monoclonal antibody" in its various grammatical forms refers to a population of antibody molecules that contain only one species of antibody combining site capable of immunoreacting with a particular epitope. A monoclonal antibody thus typically displays a single binding affinity for any epitope with which it immunoreacts. A monoclonal antibody may therefore contain an antibody molecule having a plurality of antibody combining sites, each immunospecific for a different epitope, e.g., a  
25 bispecific monoclonal antibody.

30 A monoclonal antibody is typically composed of antibodies produced by clones of a single cell called a hybridoma that secretes (produces) but one kind of antibody molecule. The hybridoma cell is formed by fusing an antibody-producing cell and a myeloma or other self-perpetuating cell line. The preparation of such antibodies was first described by Kohler and Milstein, Nature 256:495-497 (1975), which description is incorporated by reference. The hybridoma  
35 supernates so prepared can be screened for the presence of antibody molecules that immunoreact with a

polypeptide of this invention, or for inhibition of the natural function of an  $\alpha_{6B}$  or  $\alpha_{3B}$  subunit.

Briefly, to form the hybridoma from which the monoclonal antibody composition is produced, a myeloma or other self-perpetuating cell line is fused with lymphocytes obtained from the spleen of a mammal hyperimmunized with an antigen containing the cytoplasmic domain of  $\alpha_{6B}$  or  $\alpha_{3B}$ , such as is present in a polypeptide of this invention. The polypeptide-induced hybridoma technology is described by Niman et al., Proc. Natl. Sci., U.S.A., 80:4949-4953 (1983), which description is incorporated herein by reference.

It is preferred that the myeloma cell line used to prepare a hybridoma be from the same species as the lymphocytes. Typically, a mouse of the strain 129 G1X<sup>+</sup> is the preferred mammal. Suitable mouse myelomas for use in the present invention include the hypoxanthine-aminopterin-thymidine-sensitive (HAT) cell lines P3X63-Ag8.653, and Sp2/0-Ag14 that are available from the American Type Culture Collection, Rockville, MD, under the designations CRL 1580 and CRL 1581, respectively.

Splenocytes are typically fused with myeloma cells using polyethylene glycol (PEG) 1500. Fused hybrids are selected by their sensitivity to HAT. Hybridomas producing a monoclonal antibody of this invention are identified using an immunoassay such as the immunoprecipitation protocol described in Example 3.

A monoclonal antibody of the present invention can also be produced by initiating a monoclonal hybridoma culture comprising a nutrient medium containing a hybridoma that secretes antibody molecules of the appropriate polypeptide specificity. The culture is maintained under conditions and for a time period sufficient for the hybridoma to secrete

the antibody molecules into the medium. The antibody-containing medium is then collected. The antibody molecules can then be further isolated by well known techniques.

5 Media useful for the preparation of these compositions are both well known in the art and commercially available and include synthetic culture media, inbred mice and the like. An exemplary synthetic medium is Dulbecco's minimal essential medium (DMEM; Dulbecco et al., Virol. 8:396 (1959)) supplemented with 4.5 gm/l glucose, 20 mm glutamine, and 20% fetal calf serum. An exemplary inbred mouse strain is the Balb/c.

10 15 The monoclonal antibodies of this invention can be used in the same manner as disclosed herein for antibodies of the present invention.

20 For example, the monoclonal antibody can be used in the diagnostic methods and systems disclosed herein where formation of a cytoplasmic  $\alpha_{6B}$  or  $\alpha_{3B}$  domain-containing immunoreaction product is desired.

25 Other methods of producing a monoclonal antibody, a hybridoma cell, or a hybridoma cell culture are also well known. See, for example, the method of isolating monoclonal antibodies from an immunological repertoire as described by Sastry, et al., Proc. Natl. Acad. Sci., 86:5728-5732 (1989); Huse et al., Science, 246:1275-1281 (1989); and Mullinax et al., Proc. Natl. Acad. Sci. USA, 87:8095-8099 (1990).

30 Also contemplated by this invention is the hybridoma cell, and cultures containing a hybridoma cell that produce a monoclonal antibody of this invention.

#### D. Diagnostic Systems

35 The present invention also describes a diagnostic system, preferably in kit form, for assaying for the presence of antigen having the cytoplasmic domain of

5        $\alpha_{6B}$  or  $\alpha_{3B}$  in a body sample such as a tissue, body fluid or the like body sample. A diagnostic system includes, in an amount sufficient for at least one assay, a subject polypeptide and/or a subject antibody or monoclonal antibody, as a separately packaged immunochemical reagent. Instructions for use of the packaged reagent are also typically included.

10       As used herein, the term "package" refers to a solid matrix or material such as glass, plastic, paper, foil and the like capable of holding within fixed limits a polypeptide, polyclonal antibody or monoclonal antibody of the present invention. Thus, for example, a package can be a glass vial used to contain milligram quantities of a contemplated polypeptide or it can be a microtiter plate well to which microgram quantities of a contemplated polypeptide have been operatively affixed, i.e., linked so as to be capable of being immunologically bound by an antibody.

15

20       "Instructions for use" typically include a tangible expression describing the reagent concentration or at least one assay method parameter such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions and the like.

25

30       In one embodiment, a diagnostic system for assaying for the presence of or to quantitate  $\alpha_{6B}$  or  $\alpha_{3B}$  in a sample, such as blood, plasma or serum, comprises a package containing at least one  $\alpha_{6B}$  or  $\alpha_{3B}$  derived polypeptide of this invention depending on whether  $\alpha_{6B}$  or  $\alpha_{3B}$  is to be detected, respectively. In another embodiment, a diagnostic system of the present invention for assaying for the presence or amount of  $\alpha_{6B}$  or  $\alpha_{3B}$  in a sample further includes an antibody

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composition of this invention. An exemplary diagnostic system is described in Example 4.

In preferred embodiments, a diagnostic system of the present invention further includes a label or 5 indicating means capable of signaling the formation of an immunocomplex containing a polypeptide or antibody molecule of the present invention.

The word "complex" as used herein refers to the product of a specific binding reaction such as an 10 antibody-antigen or receptor-ligand reaction.

Exemplary complexes are immunoreaction products.

As used herein, the terms "label" and "indicating 15 means" in their various grammatical forms refer to single atoms and molecules that are either directly or indirectly involved in the production of a detectable signal to indicate the presence of a complex. Any label or indicating means can be linked to or incorporated in an expressed protein, polypeptide, or antibody molecule that is part of an antibody or 20 monoclonal antibody composition of the present invention, or used separately, and those atoms or molecules can be used alone or in conjunction with additional reagents. Such labels are themselves well-known in clinical diagnostic chemistry and constitute 25 a part of this invention only insofar as they are utilized with otherwise novel proteins methods and/or systems.

The labeling means can be a fluorescent labeling 30 agent that chemically binds to antibodies or antigens without denaturing them to form a fluorochrome (dye) that is a useful immunofluorescent tracer. Suitable fluorescent labeling agents are fluorochromes such as fluorescein isocyanate (FIC), fluorescein isothiocyanate (FITC), 5-dimethylamine-1-naphthalenesulfonyl chloride (DANSC), 35 tetramethylrhodamine isothiocyanate (TRITC),

lissamine, rhodamine 8200 sulphonyl chloride (RB 200 SC) and the like. A description of immunofluorescence analysis techniques is found in DeLuca, "Immunofluorescence Analysis", in Antibody As a Tool, 5 Marchalonis, et al., eds., John Wiley & Sons, Ltd., pp. 189-231 (1982), which is incorporated herein by reference.

In preferred embodiments, the indicating group is an enzyme, such as horseradish peroxidase (HRP), 10 glucose oxidase, or the like. In such cases where the principal indicating group is an enzyme such as HRP or glucose oxidase, additional reagents are required to visualize the fact that a receptor-ligand complex 15 (immunoreactant) has formed. Such additional reagents for HRP include hydrogen peroxide and an oxidation dye precursor such as diaminobenzidine. An additional reagent useful with glucose oxidase is 2,2'-azino-di-(3-ethyl-benzthiazoline-G-sulfonic acid) (ABTS).

Radioactive elements are also useful labeling 20 agents and are used illustratively herein. An exemplary radiolabeling agent is a radioactive element that produces gamma ray emissions. Elements which 25 themselves emit gamma rays, such as  $^{124}\text{I}$ ,  $^{125}\text{I}$ ,  $^{128}\text{I}$ ,  $^{132}\text{I}$  and  $^{51}\text{Cr}$  represent one class of gamma ray emission-producing radioactive element indicating groups. Particularly preferred is  $^{125}\text{I}$ . Another group of 30 useful labeling means are those elements such as  $^{11}\text{C}$ ,  $^{18}\text{F}$ ,  $^{15}\text{O}$  and  $^{13}\text{N}$  which themselves emit positrons. The positrons so emitted produce gamma rays upon encounters with electrons present in the animal's body. Also useful is a beta emitter, such  $^{111}\text{indium}$  of  $^{3}\text{H}$ .

The linking of labels, i.e., labeling of, 35 polypeptides and proteins is well known in the art. For instance, antibody molecules produced by a hybridoma can be labeled by metabolic incorporation of

radioisotope-containing amino acids provided as a component in the culture medium. See, for example, Galfre et al., Meth. Enzymol., 73:3-46 (1981). The techniques of protein conjugation or coupling through activated functional groups are particularly applicable. See, for example, Aurameas, et al., Scand. J. Immunol., Vol. 8 Suppl. 7:7-23 (1978), Rodwell et al., Biotech., 3:889-894 (1984), and U.S. Pat. No. 4,493,795.

The diagnostic systems can also include, preferably as a separate package, a specific binding agent. A "specific binding agent" is a molecular entity capable of selectively binding a reagent species of the present invention or a complex containing such a species, but is not itself a polypeptide or antibody molecule composition of the present invention. Exemplary specific binding agents are second antibody molecules, complement proteins or fragments thereof, S. aureus protein A, and the like. Preferably the specific binding agent binds the reagent species when that species is present as part of a complex.

In preferred embodiments, the specific binding agent is labeled. However, when the diagnostic system includes a specific binding agent that is not labeled, the agent is typically used as an amplifying means or reagent. In these embodiments, the labeled specific binding agent is capable of specifically binding the amplifying means when the amplifying means is bound to a reagent species-containing complex.

The diagnostic kits of the present invention can be used in an "ELISA" format to detect the quantity of  $\alpha_{6\beta}$  or  $\alpha_{3\beta}$  subunit in a vascular fluid sample such as blood, serum, or plasma. "ELISA" refers to an enzyme-linked immunosorbent assay that employs an antibody or antigen bound to a solid phase and an enzyme-antigen

5 or enzyme-antibody conjugate to detect and quantify the amount of an antigen present in a sample. A description of the ELISA technique is found in Chapter 22 of the 4th Edition of Basic and Clinical Immunology by D.P. Sites et al., published by Lange Medical Publications of Los Altos, CA in 1982 and in U.S. Patents No. 3,654,090; No. 3,850,752; and No. 4,016,043, which are all incorporated herein by reference.

10 Thus, in preferred embodiments, a polypeptide or an antibody of the present invention can be affixed to a solid matrix to form a solid support that comprises a package in the subject diagnostic systems.

15 A reagent is typically affixed to a solid matrix by adsorption from an aqueous medium although other modes of affixation applicable to proteins and polypeptides well known to those skilled in the art, can be used.

20 Useful solid matrices are also well known in the art. Such materials are water insoluble and include the cross-linked dextran available under the trademark SEPHADEX from Pharmacia Fine Chemicals (Piscataway, NJ); agarose; beads of polystyrene beads about 1 micron to about 5 millimeters in diameter available 25 from Abbott Laboratories of North Chicago, IL; polyvinyl chloride, polystyrene, cross-linked polyacrylamide, nitrocellulose- or nylon-based webs such as sheets, strips or paddles; or tubes, plates or the wells of a microtiter plate such as those made 30 from polystyrene or polyvinylchloride.

35 The reagent species, labeled specific binding agent or amplifying reagent of any diagnostic system described herein can be provided in solution, as a liquid dispersion or as a substantially dry power, e.g., in lyophilized form. Where the indicating means is an enzyme, the enzyme's substrate can also be

provided in a separate package of a system. A solid support such as the before-described microtiter plate and one or more buffers can also be included as separately packaged elements in this diagnostic assay system.

The packaging materials discussed herein in relation to diagnostic systems are those customarily utilized in diagnostic systems.

The term "package" refers to a solid matrix or material such as glass, plastic (e.g., polyethylene, polypropylene and polycarbonate), paper, foil and the like capable of holding within fixed limits a diagnostic reagent such as a polypeptide, antibody or monoclonal antibody of the present invention. Thus, for example, a package can be a bottle, vial, plastic and plastic-foil laminated envelope or the like container used to contain a contemplated diagnostic reagent or it can be a microtiter plate well to which microgram quantities of a contemplated diagnostic reagent have been operatively affixed, i.e., linked so as to be capable of being immunologically bound by an antibody or polypeptide to be detected.

#### F. Assay Methods

The present invention contemplates various immunoassay methods for determining the amount of  $\alpha_{6B}$  or  $\alpha_{3B}$  in a biological sample using a polypeptide, polyclonal antibody or monoclonal antibody of this invention as an immunochemical reagent to form an immunoreaction product whose amount relates, either directly or indirectly, to the amount of  $\alpha_{6B}$  or  $\alpha_{3B}$  in the sample.

Those skilled in the art will understand that there are numerous well known clinical diagnostic chemistry procedures in which an immunochemical reagent of this invention can be used to form an immunoreaction product whose amount relates to the

amount of  $\alpha_{6B}$  or  $\alpha_{3B}$  present in a body sample. Thus, while exemplary assay methods are described herein, the invention is not so limited.

Various heterogeneous and homogeneous protocols, either competitive or noncompetitive, can be employed in performing an assay method of this invention, including radioimmunoprecipitation (RIP), solid phase immunoassay such as ELISA, in situ immunoreaction assays for direct binding of antigen in tissue samples, and the like immunoassay protocols.

Generally, to detect the presence of an  $\alpha_{6B}$  or  $\alpha_{3B}$  subunit or polypeptide in a patient, an aliquot (i.e., a predetermined amount) of a body fluid sample, such as urine or a vascular fluid, namely blood, plasma or serum from the patient, or a tissue sample prepared for immunoreaction, is contacted by admixture (admixed), with an antibody composition of the present invention to form an immunoreaction admixture. The admixture is then maintained under biological assay conditions (immunoreaction conditions) for a period of time sufficient for the  $\alpha_{6B}$  or  $\alpha_{3B}$  antigen present in the sample to immunoreact with (immunologically bind) a portion of the antibody combining sites present in the antibody composition to form a antigen-antibody molecule immunoreaction product (immunocomplex). The complex can then be detected as described herein. The presence of the complex is indicative of  $\alpha_{6B}$  or  $\alpha_{3B}$  subunit or polypeptide in the sample.

Maintenance time periods sufficient for immunoreaction are well known and are typically from about 10 minutes to about 16-20 hours at a temperature of about 4°C to about 45°C, with the time and temperature typically being inversely related. For example, longer maintenance times are utilized at lower temperatures, such as 16 hours at 4°C, and

shorter times for higher temperatures, such as 1 hour at room temperature.

Biological assay conditions are those that maintain the biological activity of the immunochemical reagents of this invention and the  $\alpha_{6B}$  or  $\alpha_{3B}$  subunit or polypeptide sought to be assayed such that the reagents retain their ability to form an immunoreaction product. Those conditions include a temperature range of about 4°C to about 45°C, a pH value of about 5 to about 9 and an ionic strength varying from that of distilled water to that of about one molar sodium chloride. Methods for optimizing such maintenance time periods and biological assay conditions are well known in the art.

Determining the presence or amount of an  $\alpha_{6B}$  or  $\alpha_{3B}$  containing immunoreaction product formed by the above maintenance step, either directly or indirectly, can be accomplished by assay techniques well known in the art, and typically depend on the type of indicating means used.

In a direct binding assay format for detecting  $\alpha_{6B}$  or  $\alpha_{3B}$  in a tissue sample such as a tissue section, the antibody is reacted with the target antigen in situ to form the immunoreaction complex. thereafter, the immunocomplex is detected thereby indicating the presence of the antigen in the tissue. Exemplary and preferred in situ immunoassay formats are described in Example 4. Alternatively, the direct binding assay can be practiced with a fluid body sample believed to contain  $\alpha_{6B}$  or  $\alpha_{3B}$  subunits or polypeptides.

Thus, in this embodiment, the direct assay comprises the steps of:

(a) admixing a tissue sample or body fluid sample with an antibody composition of this invention 35 immunospecific for a cytoplasmic domain of either  $\alpha_{6B}$

or  $\alpha_{3B}$  as described herein to form an immunoreaction admixture;

5 (b) maintaining said immunoreaction admixture under biological assay conditions for a time period sufficient to form an immunoreaction product; and

(c) detecting the presence, and preferably amount, of the immunoreaction product formed phase in step (b), and thereby the amount of presence/amount of  $\alpha_{6B}$  or  $\alpha_{3B}$  in the sample.

10 More preferably, detecting in step (c) is performed by the steps of:

(i) admixing the immunoreaction product formed in step (b) with an indicating means to form a second reaction admixture;

15 (ii) maintaining the second reaction admixture for a time period sufficient for said indication means to bind the immunoreaction product formed in step (b) and form a second reaction product; and,

20 (iii) determining the presence and/or amount of indicating means in the second reaction product, and thereby the presence of the immunoreaction product formed in step (b). Particularly preferred is the use of a labeled second antibody, immunospecific for the first antibody, as the indicating means, and preferably the label is horseradish peroxidase. In one embodiment, it is particularly preferred to use (1) mouse anti-cytoplasmic domain  $\alpha_{6B}$  polypeptide antibody in the antibody composition, and (2) goat anti-mouse IgG antibodies labeled with horseradish peroxidase as the indicating means.

25 In a preferred competition assay method, the immunoreaction admixture described above further contains a solid phase having affixed thereto a solid phase antigen comprising an  $\alpha_{6B}$  or  $\alpha_{3B}$  subunit or polypeptide having an amino acid residue sequence that includes the cytoplasmic domain of  $\alpha_{6B}$  or  $\alpha_{3B}$ .

respectively, of this invention. Thus, in this embodiment, the assay comprises the steps of:

(a) admixing a body fluid sample with 1) an antibody composition of this invention and 2) a solid support having affixed thereto (operatively linked) an antigen comprising an  $\alpha_{6B}$  or  $\alpha_{3B}$  subunit or polypeptide having an amino acid residue sequence that includes the cytoplasmic domain of  $\alpha_{6B}$  or  $\alpha_{3B}$  of this invention, or both, to form an immunoreaction admixture having both a liquid phase and a solid phase;

(b) maintaining said immunoreaction admixture under biological assay conditions for a time period sufficient to form an immunoreaction product in the solid phase; and

(c) detecting the presence, and preferably amount, of the immunoreaction product formed in the solid phase in step (b), and thereby the amount of presence/amount of one or both of  $\alpha_{6B}$  and  $\alpha_{3B}$  in the body fluid sample.

In another competition assay format the immunoreaction admixture contains (1) a body fluid sample, preferably cell free, (2) an antibody of this invention and (3) a labeled antigen comprising the cytoplasmic domain of  $\alpha_{6B}$  or  $\alpha_{3B}$ , wherein the antibody is present in the solid phase, being affixed to a solid support, to form a liquid and a solid phase. In this embodiment, the admixed body fluid sample competes with the labeled reagent for immunoreaction with the solid phase antibody to form a solid phase immunoreaction product. Thereafter, the detection of label in the solid phase correlates with the amount of  $\alpha_{6B}$  or  $\alpha_{3B}$  in the admixed fluid sample.

In one embodiment, the detection of a polypeptide of this invention in a body sample is utilized as a means to monitor the fate of therapeutically

administered  $\alpha_{6B}$  or  $\alpha_{3B}$  derived polypeptides according to the therapeutic methods disclosed herein.

Also contemplated are immunological assays capable of detecting the presence of immunoreaction product formation without the use of a label. Such methods employ a "detection means", which means are themselves well-known in clinical diagnostic chemistry and constitute a part of this invention only insofar as they are utilized with otherwise novel polypeptides, methods and systems. Exemplary detection means include methods known as biosensors and include biosensing methods based on detecting changes in the reflectivity of a surface, changes in the absorption of an evanescent wave by optical fibers or changes in the propagation of surface acoustical waves.

#### Examples

The following Examples illustrate, but do not limit, the present invention.

##### 1. Polypeptides

Polypeptides were synthesized using the classical solid-phase technique described by Merrifield, Adv. Enzymol., 32:221-96 (1969) as adapted for use with a Model 430A automated peptide synthesizer (Applied Biosystems, Foster City, CA). Polypeptide resins were cleaved by hydrogen fluoride, extracted and analyzed for purity by high-performance liquid chromatograph using a reverse-phase C18 column. (Waters Associates, Milford, MA).

The amino acid residue sequence of the polypeptides and their designations are as follows:

$p\alpha_{6A}1$  IHAQPSDKERLTSDA

$p\alpha_{6B}1$  DEKYIDNLEKKQWITKWNRNEYS

Polypeptide  $p\alpha_{6A}$  has a sequence from the cytoplasmic domain of  $\alpha_{6A}$  and is shown in SEQ ID NO 1

from residue 1059 to residue 1073 to which an additional cysteine residue was included at the N-terminus for coupling the peptide to a protein carrier (KLH) for immunization. Polypeptide  $\text{p}\alpha_{6B}$  has a sequence from the cytoplasmic domain of  $\alpha_{6B}$  and is shown in SEQ ID NO 3 from residue 1068 to residue 1091 to which an additional cysteine residue was included at the N-terminus for coupling the peptide to a protein carrier (KLH) for immunization.

10 2. Preparation of Polyclonal Antisera

a. Conjugation of KLH

Briefly, as a generalized procedure for each polypeptide, 4 milligrams of KLH in 0.25 milliliters (ml) of 10 millimolar (mM) sodium phosphate buffer (pH 15 7.2) is reacted with 0.7 milligrams (mg) of MBS dissolved in DMF, and the resulting admixture is stirred for 30 minutes at room temperature. The MBS solution is added dropwise to ensure that the local concentration of DMF was not too high, as KLH is 20 insoluble at DMF concentrations of about 30% or higher. The reaction product, KLH-MB, is passed through a chromatography column prepared with Sephadex G-25 (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated with 50 mM sodium phosphate buffer (pH 25 6.0) to remove free MBS. KLH recovery from peak fractions of the column eluate, monitored at 280 nanometers, is typically approximately 80%.

The KLH-MB so prepared is then reacted with 5 mg 30 of polypeptide dissolved in 1 ml of buffer. The pH value of the resulting reaction composition is adjusted to 7-7.5, and the reaction composition is stirred at room temperature for 3 hours to provide a polypeptide-carrier conjugate.

35 b. Immunization and Harvesting of Polyclonal Antisera

Inoculum stock solutions are prepared with CFA, IFA or alum as follows: An amount of the synthetic polypeptide-conjugate sufficient to provide the desired amount of polypeptide per inoculation is dissolved in phosphate-buffered saline (PBS) at a pH value of 7.2. Equal volumes of CFA, IFA or alum are then mixed with the polypeptide solution to provide an inoculum containing polypeptide, water and adjuvant in which the water-to-oil ratio is about 1:1. The mixture is thereafter homogenized to provide the inoculum stock solution.

Rabbits used herein to raise anti-polypeptide antibodies were injected subcutaneously with an inoculum comprising 200 micrograms (ug) of a polypeptide conjugate (polypeptide plus carrier) emulsified in complete Freund's adjuvant (CFA); 200 ug of polypeptide conjugate, incomplete in Freund's adjuvant (IFA); and 200 ug of polypeptide conjugate with 4 mg alum injected intraperitoneally on days 0, 14 and 21, respectively, of the immunization schedule. Each inoculation (immunization) consisted of four injections of the inoculum. Mice may be immunized in a similar way using about one tenth of the above dose per injection.

Animals are typically bled 4 and 15 weeks after the first injection. Control re-immune serum was obtained from each animal by bleeding just before the initial immunization.

Control inoculum stock solutions can also be prepared with keyhole limpet hemocyanin (KLH), KLH in IFA (incomplete Freund's adjuvant), KLH-alum absorbed, KLH-alum absorbed-pertussis, edestin, thyroglobulin, tetanus toxoid, tetanus toxoid in IFA, cholera toxoid and cholera toxoid in IFA.

Upon injection or other introduction of the antigen or inoculum into the host animal, the immune

system of the animal responds by producing large amounts of antibody to the antigen. Since the specific antigenic determinant of the manufactured antigen; i.e., the antigen formed from the synthetic polypeptide linked to the carrier corresponds to the determinant of the natural antigen of interest, the host animal manufactures antibodies not only to the synthetic polypeptide to which the synthetic polypeptide antigen corresponds; i.e., to the  $\alpha_{6B}$  protein.

10 c. Immunoreactivity of Anti-peptide Antisera with Native  $\alpha_6$  Proteins

15 1. Protocols and Reagents

The rabbit polyclonal anti- $\alpha_6$  cytoplasmic domain antiserum designated 6844 was raised against the last 15 amino acids (IHAQPSDKERLTSDA) (SEQ ID NO 1, residue 1059 to residue 1073) of the reported human  $\alpha_6$  ( $\alpha_{6A}$ ) sequence (Tamura et al., J. Cell. Biol., 111:1593-1604, 1990), to which an additional cystine residue was included at the N-terminus for coupling the peptide to a protein carrier (KLH) for immunization.

20 25 The rat monoclonal antibody, GoH3, is specific for an extracellular epitope on both the human and murine  $\alpha_6$  subunits (Sonnenberg et al., J. Biol. Chem., 262:10376-83, 1987). The isotype-matched control antibody, B3B4, recognizes the B lymphocyte specific antigen, CD23.

30 35 The anti- $\alpha_6$  specific monoclonal antibody, 135.13c, and the isotype matched control antibody, 439.9b, specific for the human  $\beta_4$  integrin subunit, have been previously described (Kennel et al., J. Biol. Chem., 264:15515-21, 1989).

Anti-peptide antisera to the cytoplasmic domains of rat  $\alpha_1$ , chicken  $\alpha_3$ , human  $\alpha_4$ , human  $\alpha_5$  and human  $\beta_1$  sequences were shown to be cross-reactive with the

respective mouse  $\beta_1$  integrins by immunoprecipitation of B16F1 melanoma, STO fibroblast and MMT carcinoma murine cell lines.

5                   Antisera to the cytoplasmic domain of human  $\alpha_{6B}$  were prepared by immunizations of rabbits with the peptide p $\alpha_{6B}1$  having the sequence DEKYIDNLEKKQWITKWRNESYS (SEQ ID NO 3, residue 1068 to residue 1091) as described above to which an additional cysteine residue was included at the N-  
10 terminus for coupling the peptide to a protein carrier (KLH) for immunization. This antisera is designated 382.

15                  The ES cells and B16F1 cell line were used in these immunoreaction studies. The ES cell line, CCE (Schwartzberg et al., Science, 246:799-803, 1989) was initially cultured on murine embryonic fibroblasts (STO cells) to prevent differentiation. However, in order to study the expression and function of integrins in this ES cell line it was necessary to remove the STO cells from the culture system.  
20                  Therefore, the CCE ES cell line was subcloned into LIF (10<sup>3</sup> units/ml) (Amrad Co. Australia) containing media (DMEM; 10% FCS, 100mM  $\beta$ -mercaptoethanol, 2mM glutamine). LIF has been shown to prevent ES cell differentiation (Moreau et al., Nature, 336:690-92, 1988; Smith et al., Nature, 336:688-90, 1988; Williams et al., Nature, 336:684-687, 1988). The sublines were cultured on gelatin (0.1%) coated plates. Several subclones were expanded and continually cultured in  
25                  LIF containing media. The subline ES1 was chosen for the studies described here. ES1 cells were allowed to differentiate on gelatin (0.1%) coated plates over a period of 8-9 days in the absence of LIF.

30                  The murine B16F1 melanoma line, obtained from Dr. Ralph Reisfeld (Scripps Clinic, La Jolla, California), was derived from a C57Bl/6 melanoma and cultured in

DMEM, 5% FCS, 2mM glutamine and penicillin-streptomycin (50 IU/ml- 50ug/ml).

5 Undifferentiated ES cells (1-2 x 10<sup>7</sup> cells) were surface labeled with Na<sup>125</sup>I using the lactoperoxidase procedure (Roth, Methods Enzymol., 37(B):223-33,

10 1975). Differentiated ES cells proved to be significantly more fragile than undifferentiated ES cells and did not survive the more rigorous washing steps required during the iodination procedure.

15 Therefore, differentiated ES cells were metabolically labeled with [<sup>35</sup>S]methionine as described previously by Kajiji et al, EMBO J., 8:673-680 (1989).

20 Preparation of non-ionic detergent cell lysates, immunoprecipitations and analysis by SDS-PAGE were performed as described by Kajiji et al (1989), supra.

25 Immunoprecipitation is conducted generally by first admixing the rabbit polyclonal antisera produced above with a cell lysate and maintaining the admixture for a time period sufficient for immunocomplexes to form. Thereafter, the immunoabsorbent Pansorbin (Sigma Chemical Co., St. Louis, MO) is added to the admixture containing the immunocomplexes and maintained to allow the Pansorbin to complex with (bind) the immunocomplex. Thereafter the Pansorbin-containing bound immunocomplexes are removed from the lysate admixture by centrifugation, washed several times and the washed immunocomplexes are released from the Pansorbin and analyzed by SDS-PAGE.

30 Sequential immunoprecipitation was also performed to identify the presence of multiple immunoreactive species in a single lysate. After a first immunoprecipitation as above the lysate is retained and subjected to a second immunoprecipitation with unbound Pansorbin. The resulting lysate from the 35 second immunoprecipitation is again retained and subjected to a third immunoprecipitation with unbound

Pansorbin. Thus by the successive rounds of sequential immunoprecipitation of a lysate using the same antibody species, that lysate becomes depleted of antigen immunoreactive with that antibody species.

5        Thereafter, the depleted lysate is divided into aliquots and each aliquot is separately immunoprecipitated (re-IP or re-immunoprecipitated) using different antibodies. Antigens in the depleted lysate that immunoprecipitate with the second antibody

10      different from the depleting first antibody are not immunoreactive with the first antibody. By sequential immunoprecipitation, two non-cross reacting antigen species can be identified. As described herein, the cytoplasmic domains of  $\alpha_{6A}$  and  $\alpha_{6B}$  are not cross

15      reactive.

Mouse  $\alpha_6$

Separate immunoprecipitations were carried out on undifferentiated murine ES1 cells with antiserum 382 raised against a synthetic peptide corresponding to the sequence of the cytoplasmic tail of human  $\alpha_{6B}$ , with control preimmune serum from the same rabbit, and with antisera 6844 directed to the cytoplasmic tail of human 6A. Only antisera 382 precipitated protein bands virtually identical to those reactive with anti- $\alpha_6$  monoclonal GoH3 which is specific for both  $\alpha_{6A}$  and  $\alpha_{6B}$ . These data indicated that ES1 cells do express  $\alpha_{6B}$  protein, probably complexed with  $\beta_1$ , and antisera 382 is capable of recognizing the  $\alpha_{6B}$  protein.

30      In contrast to the immunoprecipitation data from undifferentiated ES1 cells, the anti- $\alpha_{6A}$  cytoplasmic domain polyclonal antiserum, 6844, could immunoprecipitate the  $\alpha_{6A}$  isoform from  $^{35}$ S-methionine labelled lysates obtained from differentiated ES1

35      cells. Thus, differentiation of ES1 cells is

accompanied by the induction of expression of the  $\alpha_{6A}$  isoform.

5 The absence of the  $\alpha_{6A}$  isoform in differentiated ES1 cells can be seen by immunoprecipitations using GoH3 or 6844, which is shown in Figure 1. Whereas the GoH3 antibody detects the lower molecular weight species corresponding to  $\alpha_6$ , the 6844 antibody, immunospecific for  $\alpha_{6A}$ , does not detect any  $\alpha_6$  species, indicating that the GoH3-reactive form is an isoform, 10 namely  $\alpha_{6B}$ .

Similar immunoprecipitation assays were carried out on the D3 ES cell line (see Figure 1). The D3 embryonic stem cell line was derived by Doetschman et al., *J. Embryol. Exp. Morph.*, 87:27-45, (1985). D3 15 cells were cultured in LIF containing medium as described above except that 15% FCS was used. Immunoprecipitations of [<sup>35</sup>S]methionine-labelled lysates showed that the  $\alpha_{6B}$  isoform is expressed at the protein level in both undifferentiated and 20 differentiated D3 cells while the  $\alpha_{6A}$  isoform was found only in the differentiated cells. This would suggest that the ability to switch on  $\alpha_{6A}$  expression upon differentiation may be a general property of ES cells.

25 Because the 382 antisera was raised to a human  $\alpha_{6B}$  cytoplasmic domain-derived polypeptide and yet is shown above to immunoreact with the mouse  $\alpha_{6B}$  protein, the above data also shows that an anti- $\alpha_{6B}$  antibody, whether raised to human or mouse varieties of  $\alpha_{6B}$  can 30 be used to immunoreact with both human or mouse  $\alpha_{6B}$ .

Human  $\alpha_6$

35 Antisera 382 to a synthetic peptide corresponding to the last 25 residues of human  $\alpha_{6B}$  immunoprecipitated from radiolabeled detergent lysates of the human choriocarcinoma cell line JAR (see Example 4 for description of JAR cells) a pattern of

bands similar or identical to those obtained with 6844, an anti-peptide antiserum to the  $\alpha_{6A}$  cytoplasmic domain, and GoH3, a monoclonal antibody to the extracellular domain of  $\alpha_6$  (see Figure 2). The bands corresponded in molecular weight to  $\alpha_6$ ,  $\beta_1$  and  $\beta_4$ , and were positively identified as such with specific antibodies. This result is compatible with JAR cells expressing both  $\alpha_6\beta_1$  and  $\alpha_6\beta_4$  heterodimers, and with PCR amplifications detecting both  $\alpha_{6A}$  and  $\alpha_{6B}$  isoform bands in JAR cells (see Example 3).

Sequential immunoprecipitations (Figure 3) showed that antibody GoH3 completely depleted the JAR lysates of antigen reactive with antisera 382 (anti- $\alpha_{6B}$ ) or 6844 (anti- $\alpha_{6A}$ ). The 382 antiserum did not remove any material reactive with 6844, and 6844 did not remove any 382-reactive material, while both antisera reduced, but did not completely remove GoH3 reactivity (Figure 2). These results indicate that JAR cells express both  $\alpha_{6A}$  and  $\alpha_{6B}$  proteins, each of which is paired with either  $\beta_1$  or  $\beta_4$ . These results also indicate that antisera raised to a mouse protein, namely the cytoplasmic domain of mouse  $\alpha_{6B}$ , immunoreacts with its human counterpart protein, human  $\alpha_{6B}$ .

25

3. Identification and Cloning of  $\alpha_{6B}$  and  $\alpha_{3B}$  cDNAs

cDNA molecules encoding human and mouse  $\alpha_{6B}$  and mouse  $\alpha_{3B}$  cytoplasmic regions were prepared and fragments of the cDNA molecules were selectively amplified using the polymerase chain reaction (PCR) in the presence of specific oligonucleotide primers in order to characterize gene expression of the  $\alpha_{6B}$  and  $\alpha_{3B}$  proteins.

35

a. Protocols

Poly-A+ mRNA was isolated from human JAR cells (American Tissue Type Collection, ATCC, Bethesda, MD, ATCC HTB 144), human U937 cells (ATCC CRL 1591), human FG cells (Dr. P. Meitner, Brown University) and both differentiated and undifferentiated cell lines using the Invitrogen Fastrack Kit (Invitrogen, La Jolla, Ca.). Single stranded cDNA was synthesized from 10 ug of mRNA using AMV reverse transcriptase (20U; Molecular Genetics Resources, Tampa, Fl.) and one ug of random hexamer primers (Pharmacia). The cDNAs were extracted with phenol/chloroform, then ethanol precipitated and about 0.5 to 10 ug cDNA was resuspended in 50-70ul of sterile water.

One ul of the resuspended cDNA was amplified per 50 ul PCR reaction mixture (2.5mM MgCl<sub>2</sub>, 50mM KCl, 10mM  $\beta$ -mercaptoethanol, 66mM Tris.HCl; pH8.3) using 0.1 uM oligonucleotide primers, 0.25mM each of dATP, dTTP, dCTP, and dGTP, and 1.25U of TAQ 1 polymerase (AmpliTaq; Perkin Elmer/Cetus, Ca.). The PCR program consisted of 2 steps: (a) 40 cycles of 1 min at 94°C, 2 min at 55°C, and 3 min at 72°C with a 5 sec/cycle extension on the 72°C segment, (b) 10 min at 72°C and a final shift to 4°C. Second round PCR was carried out on one ul of the reaction mixture generated from the first round PCR.

Nested pairs of PCR primers were employed to ensure that  $\alpha_6$  specific fragments were amplified. Both sets of  $\alpha_6$  primers were derived from the human  $\alpha_6\beta_1$  cDNA sequence as determined by Tamura et al., J. Cell. Biol., 111:1593-1604, (1990). The first set corresponded to bp 2918-2937 (primer 1157) and 3454-3473 (primer 1156) of the human  $\alpha_6\beta_1$  sequence while the nested primer pair corresponded to bp 2942-2960 (primer 1681) and 3433-3452 (primer 2002). The sequence of these four primers are shown in SEQ ID NOS

11-14, respectively.  $\alpha_6$  PCR primers designated primer 2032 and 2033 were derived from the hamster cDNA sequence as determined by Tsuji et al., J. Biol. Chem., 265:7016-7021 (1990). The sequence of primers 5 2032 and 2033 are shown in SEQ ID NOS 15 and 16, respectively.

Oligonucleotide primers were chemically synthesized by using a "Gene Assembler" automated synthesizer (Pharmacia, Piscataway, NJ).

10 Amplified fragments from first round PCR were purified using Gene Clean (Bio 101, La Jolla, Ca), treated with DNA polymerase I and T4 polynucleotide kinase, again purified with Gene Clean, and the blunt-ended fragments were subcloned into the EcoRV site of 15 Bluescript-pKS+ (Stratagene, La Jolla, Ca). Clones containing insert were sequenced manually (Sequenase kit; USB, Cleveland, Oh) according to the manufacturer's instructions using T3 and T7 polymerase vector primer sequences. Sequences were analyzed on a 20 VAX-VMS, version 5.2 computer, with programs of the University of Wisconsin Genetics Computer Group (Devereux et al, Nucl. Acids Res., 12:387-395, 1984).

b. Results

25 i. Human  $\alpha_6$

Oligonucleotides 1156 and 1157 flanking the 3' end of the coding region of integrin  $\alpha_6$  mRNA were used as primers in polymerase chain reactions (PCR). Two products, of 540 bp and 410 bp, were 30 obtained using first strand cDNAs from various cell lines as templates (Figure 4). These same products were obtained in second-round PCR with a nested set of primers, indicating their specificity.

Both the 540bp and the 410bp PCR 35 products were subcloned and sequenced. The nucleotide sequence of the 540 bp fragment (designated  $\alpha_6A$  in

Figure 5) matches exactly the sequence of  $\alpha_6$  mRNA recently reported by Tamura et al., J. Cell. Biol., 111:1593-1604, 1990, and encodes the 3' portion of the end of the extracellular domain, the transmembrane and the cytoplasmic domains, followed by the initial part 5 of the 3' untranslated region (3' UT).

The sequence of the 410 bp band matches the 540 bp sequence, with the exception of a 130 bp gap shown in the lower sequence of Figure 5, which 10 lower sequence corresponds to the nucleotide sequence of  $\alpha_{6B}$ . This gap corresponds to the region encoding the predicted  $\alpha_{6A}$  cytoplasmic domain, from the boundary with the transmembrane domain to 25 bp past the stop codon. Without this 130 bp segment, however, 15 the open reading frame continues in the previous 3' UT, resulting in an  $\alpha_{6B}$  protein with an alternative cytoplasmic domain (Figure 6). This alternative domain is 17 amino acid longer than, and bears no sequence homology with, the reported  $\alpha_{6A}$  cytoplasmic domain, 20 but it does contain the sequence GFFKR, a motif present at the upstream border of all mammalian integrin  $\alpha$  chains sequenced. For convenience, the published  $\alpha_6$  sequence is referred to as  $\alpha_{6A}$ , and the  $\alpha_6$  having the isoform cytoplasmic domain identified 25 herein is referred to as  $\alpha_{6B}$ .

ii. Mouse  $\alpha_6$

Amplification of mouse  $\alpha_6$  cDNA expressed by undifferentiated ES1 and B16F1 cells was performed on first strand cDNA derived from these 30 mouse cells using the polymerase chain reaction (PCR). The nested sets of PCR primers, pairs 1157/1156 and 1681/2002 described above, were employed. Figure 7A shows the PCR products obtained.

The PCR fragment amplified from B16F1 ("B16") 35 cDNA correspond to the size expected (510 bp) for the murine homologue of the human  $\alpha_6$  (Figure 7A; lane 2).

However, the PCR fragment obtained from the amplification of the ES1 cell cDNA was significantly smaller. Amplification of cDNAs derived from four independent ES1 mRNA preparations yielded only the smaller fragment and never the larger fragment amplified from B16F1 cDNA.

The PCR fragments from the ES1 and B16F1 cells were subcloned into the Bluescript-pKS+ vector and sequenced. Figure 8 shows the nucleotide sequences of the two PCR fragments. The sequence of the larger B16F1 fragment was shown to be 89% identical to the human  $\alpha_{6A}$  sequence at the nucleotide level and 91% identical at the amino acid level, Tamura et al., *J. Cell. Biol.*, 111:1593-1604 (1990). Thus the larger fragment's sequence represents the murine homologue of the human  $\alpha_{6A}$  subunit. The B16F1 PCR fragment (Figure 8) encodes the C-terminal portion of the extracellular domain as well as the transmembrane and cytoplasmic domains of the  $\alpha_6$  subunit. Due to the selection of primers, additional coding sequences 3' to the terminus of the sequence shown in Figure 8 were not detected. Thus, additional amino acid residues not shown in Figure 8 are present in the native mouse  $\alpha_{6B}$  protein.

The sequence of the smaller PCR fragment (Figure 8) was identical to the B16F1 sequence except that an internal deletion of 130 bp was observed. The location of the 130 bp deletion observed in the ES1  $\alpha_6$  PCR fragment exactly matched that of the human  $\alpha_{6B}$  sequence. Therefore, ES1 cells expressed the murine equivalent of the  $\alpha_{6B}$  isoform.

### iii. Mouse $\alpha_6$

Expression of the  $\alpha_6$  isoforms was also investigated in various mouse tissues including muscle, heart, brain, lung and ovary. Using the PCR procedure described above with the hamster  $\alpha_6$  primers,

a larger band corresponding to  $\alpha_{3A}$  was amplified from most tissues except heart, kidney, liver, thymus and spleen (Table 2; Example 4). A smaller band corresponding to  $\alpha_{3B}$  was detected in heart and brain. 5 Cloning and sequencing of these bands showed that the larger band corresponds exactly to the reported  $\alpha_3$  sequence ( $\alpha_{3A}$ ), while the smaller band lacks a 144 bp segment and, like  $\alpha_{6B}$ , encodes an  $\alpha_3$  with an alternative cytoplasmic tail ( $\alpha_{3B}$ ). The amino acid 10 residue and nucleotide sequences of the mouse  $\alpha_{3B}$  cDNA-derived PCR fragments are shown in SEQ ID NGS 9 and 10, respectively.

4. Tissue distribution of  $\alpha_{6A}$ ,  $\alpha_{6B}$ ,  $\alpha_{3A}$  and  $\alpha_{3B}$

15 a. PCR Amplification

The distribution of the  $\alpha_6$  isoforms in cultured cell lines and mouse tissue was assessed by PCR as described in Example 3. The majority of the cells tested contained both  $\alpha_{6A}$  and  $\alpha_{6B}$  mRNA (see 20 Tables 1 and 2). However, the two isoforms were reproducibly found at ratios characteristic of a cell line. Interestingly, two carcinoma cell lines and three lines of mouse embryonic fibroblasts (immortalized, non-transformed) contained exclusively 25  $\alpha_{6A}$ , while embryonic stem cells and F9 teratocarcinoma cells contained exclusively  $\alpha_{6B}$  (Table 1).

TABLE 1

| 30 | <u>CELL LINE</u> | <u>CELL TYPE</u>     | $\alpha_{6A}$ | $\alpha_{6B}$ | $\alpha_{3A}$ | $\alpha_{3B}$ |
|----|------------------|----------------------|---------------|---------------|---------------|---------------|
|    | FG               | Pancreatic Carcinoma | +             | >             | +             | +             |
|    | 1320 Met         | "                    | +             | >             | +             | ND            |
|    | Panc-1           | "                    | +             | >             | +             | -             |
|    | SGR              | "                    | +             | >             | +             | ND            |
| 35 | JAR              | Choriocarcinoma      | +             | <             | +             | -             |
|    | JEG-3            | "                    | +             | <             | +             | ND            |

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|    |           |                      |   |   |   |    |    |
|----|-----------|----------------------|---|---|---|----|----|
|    | BeWo      | "                    | + | < | + | ND | ND |
|    | LoVo      | Colon Carcinoma      | + | < | + | ND | ND |
|    | Colo 396  | "                    | + | < | + | +  | -  |
|    | CaCo-2    | "                    | + |   | + | +  | -  |
| 5  | HT-29     | "                    | + | > | + | +  | -  |
|    | HeLa      | Cervical Carcinoma   | + | > | + | ND | ND |
|    | UCLA-P3   | Lung Carcinoma       | + | > | + | +  | -  |
|    | A431      | Epidermoid Carcinoma | + |   | - | ND | ND |
|    | K562      | Erythroleukemia      | + |   | + | ND | ND |
| 10 | U937      | Histiocytic Lymphoma | + | > | + | +  | -  |
|    | 804G(Rat) | Bladder Carcinoma    | + |   | - | +  | -  |
|    | 3T3 (M)   | Embryonic Fibroblast | + |   | - | +  | -  |
|    | F9 (M)    | Teratocarcinoma      | - |   | + | +  | -  |
|    | ES (M)    | Embryonic Stem       | - |   | + | +  | -  |
| 15 | ES (M)    | (Differentiated)     | + |   | + | ND | ND |

Cells were analyzed by PCR amplification of  $\alpha_6$  and  $\alpha_5$  isoforms using the following human or mouse cells, with the cell sources indicated in parenthesis: pancreatic carcinoma: 20 FG, SGR and 1320 Met cells (Dr. P. Meitner, Brown University); Panc-1 cells (ATCC CRL 1469); choriocarcinoma: JAR cells (ATCC HTB 144); JEG-3 cells (ATCC HTB 36); BeWo cells (ATCC CCL 98); colon carcinoma: LoVo cells (ATCC CCL 229); Colo 396 cells (Dr. T. Edgington, Scripps Clinic, La Jolla, CA); CaCo-2 cells (ATCC HTB 37); HT-29 cells (ATCC HTB 38); Hela cervical carcinoma cells (ATCC CCL 2); UCLA-P3 lung carcinoma cells (L. Walker, Scripps); A431 epidermoid carcinoma cells (ATCC CRL 1555); K562 erythroleukemia cells (ATCC CCL 243); U937 histiocytic lymphoma cells (ATCC CCL 1593); 8049 rat bladder carcinoma cells (J. Jones, Northwestern University, Evanston, IL); NIH/3T3 mouse embryo fibroblasts (ATCC CRL 1658); F9 mouse teratocarcinoma cells ATCC CRL 1720); ES mouse embryonic stem cells (E. Robertson, Columbia University, NY).

Table 1 illustrates the distinction of  $\alpha_{6A}$  and  $\alpha_{6B}$ , and  $\alpha_{3A}$  and  $\alpha_{3B}$  subunit-encoding mRNAs in human and mouse cultured cell lines. PCR amplification was performed on single-stranded cDNA generated from each cell type, using oligonucleotides specific for the  $\alpha_6$  or  $\alpha_3$  subunit, respectively. The (+) symbol represents the presence of subunit-specific amplification product in the tested sample, the (-) symbol represents its absence, and (ND) indicates that analysis was not conducted on that tissue type. The (>) is used when the  $\alpha_{6A}$  subunit mRNA predominates over the  $\alpha_{6B}$  subunit mRNA, and the (<) symbol is used when the  $\alpha_{6B}$  subunit mRNA is the predominant species in the tissue.

By the same PCR assay, normal mouse lung, liver, spleen and cervix tissues were solely  $\alpha_{6A}$ , brain, ovary and kidney were solely  $\alpha_{6B}$ , while all other tissues tested contained both  $\alpha_6$  isoforms (Table 2).

TABLE 2

20

| <u>TISSUE</u> | <u><math>\alpha_{6A}</math></u> | <u><math>\alpha_{6B}</math></u> | <u><math>\alpha_{3A}</math></u> | <u><math>\alpha_{3B}</math></u> |
|---------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Muscle        | +                               | >                               | +                               | -                               |
| Heart         | +                               | >                               | +                               | +                               |
| Kidney        | -                               | +                               | -                               | -                               |
| 25 Liver      | +                               | -                               | -                               | -                               |
| Brain         | -                               | +                               | +                               | <                               |
| Lung          | +                               | -                               | +                               | -                               |
| Stomach       | +                               | <                               | +                               | ND                              |
| Intestine     | +                               | <                               | +                               | ND                              |
| 30 Cervix     | +                               | -                               | ND                              | ND                              |
| Submax        | +                               | +                               | ND                              | ND                              |
| Ovary         | -                               | +                               | +                               | -                               |
| Thymus        | +                               | >                               | -                               | -                               |
| Spleen        | +                               | -                               | -                               | -                               |

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Table 2 illustrates the distribution of  $\alpha_{6A}$  and B and  $\alpha_{3A}$  and B subunit-encoding mRNAs in mouse tissues. The symbols in Table 2 are the same as in Table 1.

5 Primary and nested PCR reactions were carried out on differentiated cell lines as described in Example 3. ES1 cells were allowed to differentiate over a period of 8-9 days in the absence of Leukemia Inhibitory Factor (LIF). The morphology of the differentiated cells was dramatically different from that of undifferentiated ES1 cells maintained in LIF. 10 PCR amplification on cDNA from undifferentiated ES1 cells, using  $\alpha_6$  specific primers, produced the 380 bp fragment corresponding to the  $\alpha_{6B}$  cytoplasmic sequence (Figure 7B, lane 1). However, similar amplification of cDNA from the differentiated cells produced two 15 distinct fragments of 510 bp and 380 bp (Figure 7B, lane 2), shown by nucleotide sequencing to be the  $\alpha_{6A}$  and  $\alpha_{6B}$  isoforms, respectively.

20 b. In Situ Immunostaining of Tissues to Detect Tissue Distribution

Kidney biopsy materials were obtained by percutaneous needle biopsies using modified Vim-Silverman needles in patients with glomerulonephritis. 25 A small portion of kidney biopsy materials were fixed with 4% paraformaldehyde for 4 hours at 4°C and embedded in paraffin using an automatic processor (Tissue-Tek<sup>®</sup> Rotary Tissue Processor). The tissue was cut in 4 micron thickness with an AO rotary microtome, and deparafinized with xylene or HistoClear (Baxter) 30 and rehydrated with graded alcohol. The rehydrated sections were washed with 0.1 M glycine in TBS (0.005 M Tris-HCl; 0.9% NaCl, pH 7.5) for 5 minutes, treated with 0.1% Triton X-100 for 2 min at room temperature (RT), and trypsinized (0.1% trypsin for 5 min at 35 37°C). Nonspecific binding sites were saturated by a

blocking solution (5% dry milk solids, 1% heat inactivated horse serum in TBS) for 30 minutes. Serially diluted primary antibodies [1 ug/ul of 33% saturated ammonium sulfate (SAS) cut of antisera 6844 and 382; 1:10 to 1:1000 dilutions in reagent diluent: i.e. 2.5% bovine serum albumin in TBS] and normal control (SAS cut of normal rabbit serum, 1ug/ul, 1:10 to 1:1000 dilution in reagent diluent) were incubated on sections in humidified chambers at room temperature for 1 hr. Tissue sections were further incubated with peroxidase conjugated goat anti-rabbit antibody (Jackson Immunology) in reagent diluent (1:200) for 30 minutes. After this, 0.02% AEC (3-Amino- $\alpha$ -ethylcarbozole, Aldrich) was applied for 30 minutes at room temperature. Each step was followed by 3-minute washes in 0.005 M Tris-HCl, 0.9% NaCl pH 7.5 (TBS wash). Washed tissue sections were counterstained with Mayor's hematoxylin for 30 sec, mounted in Gel Mount (Biomedica) and observed under a light microscope.

The results of the in situ immunostained kidney sections using the anti-peptide antisera specific for  $\alpha_{6A}$  (6844) or specific for  $\alpha_{6B}$  (382) are shown in Figure 9. Panel A shows anti- $\alpha_{6A}$  antibody molecules staining podocytes in the glomerular structure of the kidney but no staining in the tubules of the kidney. Panel B shows anti- $\alpha_{6B}$  antibody molecules staining the epithelial cells of the distal or collecting tubules of the kidney but not the glomerular cells.

Additional kidney samples were similarly analyzed and the results are shown in Table 3.

TABLE 3<sup>1</sup>

|    | <u>Patient</u> | <u>Sera 6488</u> |          | <u>Sera 382</u> |          |
|----|----------------|------------------|----------|-----------------|----------|
|    |                | <u>G</u>         | <u>T</u> | <u>G</u>        | <u>T</u> |
| 5  | Normal         | -                | -        | -               | -        |
|    | 1              | +                | -        | -               | +        |
|    | 2              | ++               | ±        | -               | ++       |
| 10 | 3              | +++              | -        | -               | +++      |
|    | 4              | +++              | -        | -               | ++++     |
|    | 5              | ++++             | -        | -               | ++++     |

<sup>1</sup> "G" indicates the immunostaining pattern observed in glomerular epithelial cells of the kidney, whereas "T" indicates the immunostaining pattern observed in the tubular epithelial cells, where (-) indicates no staining and + to ++++ indicates increasing intensities of stain. Patients 1-5 are patients that all have glomerular nephritis clinically indicated as to require kidney biopsy.

The results in Table 3 indicate that in all patients exhibiting symptoms of kidney dysfunction a distinct staining pattern is observed, namely that antisera immunospecific for  $\alpha_{6A}$  cytoplasmic domain (6488) reacts with glomerular cells and antisera immunospecific for 2246B cytoplasmic domain (382) reacts with the tubular epithelial cells.

These results show that antibodies immunoreactive with the cytoplasmic domain of  $\alpha_{6B}$  are useful for distinguishing cell types in kidney sections, and particularly to identify distal and collecting tubular epithelial cells in patients having conditions of kidney dysfunction such as glomerular nephritis.

35 Although the present invention has now been described in terms of certain preferred embodiments,

and exemplified with respect thereto, one skilled in the art will readily appreciate that various modifications, changes, omissions and substitutions may be made without departing from the spirit thereof.

5 It is intended, therefore, that the present invention be limited solely by the scope of the following claims.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: Tamura, Richard N.  
Quaranta, Vito

(ii) TITLE OF INVENTION: INTEGRIN ALPHA SUBUNIT CYTOPLASMIC  
DOMAIN POLYPEPTIDES, ANTIBODIES AND METHODS

(iii) NUMBER OF SEQUENCES: 16

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(F) ZIP: 92037

## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
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(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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## (viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Fitting, Thomas  
(B) REGISTRATION NUMBER: 34,163  
(C) REFERENCE/DOCKET NUMBER: BEC001OP

## (ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 619-554-2937

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1073 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Homo sapiens*

(ix) FEATURE:

(A) NAME/KEY: Domain

(B) LOCATION: 1012..1037

(D) OTHER INFORMATION: /label= TRANSMEMBRANE  
/note= "The putative transmembrane region is  
encompassed by amino acids 1012-1037."

(ix) FEATURE:

(A) NAME/KEY: Cleavage-site

(B) LOCATION: (23^24)

(D) OTHER INFORMATION: /note= "The mature protein is  
cleaved from the signal sequence between amino  
acids 23-24."

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 223

(D) OTHER INFORMATION: /label= GLYCOSYLATION  
/note= "Potential site of N-linked glycosylation."

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 284

(D) OTHER INFORMATION: /label= GLYCOSYLATION  
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(B) LOCATION: 370

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/note= "Potential site of N-linked glycosylation."

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 513

(D) OTHER INFORMATION: /label= GLYCOSYLATION  
/note= "Potential site of N-linked glycosylation."

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(A) NAME/KEY: Modified-site

(B) LOCATION: 731

(D) OTHER INFORMATION: /label= GLYCOSYLATION  
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(B) LOCATION: 748  
(D) OTHER INFORMATION: /label= GLYCOSYLATION  
/note= "Potential site of N-linked glycosylation."

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(A) NAME/KEY: Modified-site  
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(D) OTHER INFORMATION: /label= GLYCOSYLATION  
/note= "Potential site of N-linked glycosylation."

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(A) NAME/KEY: Modified-site  
(B) LOCATION: 927  
(D) OTHER INFORMATION: /label= GLYCOSYLATION  
/note= "Potential site of N-linked glycosylation."

## (ix) FEATURE:

(A) NAME/KEY: Modified-site  
(B) LOCATION: 958  
(D) OTHER INFORMATION: /label= GLYCOSYLATION  
/note= "Potential site of N-linked glycosylation."

## (ix) FEATURE:

(A) NAME/KEY: Binding-site  
(B) LOCATION: 230..238  
(D) OTHER INFORMATION: /note= "Represents a putative cation binding domain."

## (ix) FEATURE:

(A) NAME/KEY: Binding-site  
(B) LOCATION: 324..332  
(D) OTHER INFORMATION: /note= "Represents a putative cation binding domain."

## (ix) FEATURE:

(A) NAME/KEY: Binding-site  
(B) LOCATION: 386..394  
(D) OTHER INFORMATION: /note= "Represents a putative cation binding domain."

## (ix) FEATURE:

(A) NAME/KEY: Binding-site  
(B) LOCATION: 441..449  
(D) OTHER INFORMATION: /note= "Represents a putative cation binding domain."

## (ix) FEATURE:

(A) NAME/KEY: Domain  
(B) LOCATION: 1040..1044

(D) OTHER INFORMATION: /label= CYTOPLASMIC  
/note= "The cytoplasmic sequence, which is  
conserved in virtually all of the integrin ALPHA  
chains."

## (ix) FEATURE:

(A) NAME/KEY: Region  
(B) LOCATION: 927..1073

(D) OTHER INFORMATION: /note= "The sequence encoded by the  
fragment of ALPHA 6A cDNA amplified using primers  
1156/1157."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Ala Ala Ala Gly Gln Leu Cys Leu Leu Tyr Leu Ser Ala Gly Leu  
1 5 10 15

Leu Ser Arg Leu Gly Ala Ala Phe Asn Leu Asp Thr Arg Glu Asp Asn  
20 25 30

Val Ile Arg Lys Tyr Gly Asp Pro Gly Ser Leu Phe Gly Phe Ser Leu  
35 40 45

Ala Met His Trp Gln Leu Gln Pro Glu Asp Lys Arg Leu Leu Leu Val  
50 55 60

Gly Ala Pro Arg Gly Glu Ala Leu Pro Leu Gln Arg Ala Phe Arg Thr  
65 70 75 80

Gly Gly Leu Tyr Ser Cys Asp Ile Thr Ala Arg Gly Pro Cys Thr Arg  
85 90 95

Ile Glu Phe Asp Asn Asp Ala Asp Pro Thr Ser Glu Ser Lys Glu Asp  
100 105 110

Gln Trp Met Gly Val Thr Val Gln Ser Gln Gly Pro Gly Gly Lys Val  
115 120 125

Val Thr Cys Ala His Arg Tyr Glu Lys Arg Gln His Val Asn Thr Lys  
130 135 140

Gln Glu Ser Arg Asp Ile Phe Gly Arg Cys Tyr Val Leu Ser Gln Asn  
145 150 155 160

Leu Arg Ile Glu Asp Asp Met Asp Gly Gly Asp Trp Ser Phe Cys Asp  
165 170 175

Gly Arg Leu Arg Gly His Glu Lys Phe Gly Ser Cys Gln Gln Gly Val  
180 185 190

70

Ala Ala Thr Phe Thr Lys Asp Phe His Tyr Ile Val Phe Gly Ala Pro  
195 200 205

Gly Thr Tyr Asn Trp Lys Gly Ile Val Arg Val Glu Gln Lys Asn Asn  
210 215 220

Thr Phe Phe Asp Met Asn Ile Phe Glu Asp Gly Pro Tyr Glu Val Gly  
225 230 235 240

Gly Glu Thr Glu His Asp Glu Ser Leu Val Pro Val Pro Ala Asn Ser  
245 250 255

Tyr Leu Gly Phe Ser Leu Asp Ser Gly Lys Gly Ile Val Ser Lys Asp  
260 265 270

Glu Ile Thr Phe Val Ser Gly Ala Pro Arg Ala Asn His Ser Gly Ala  
275 280 285

Val Val Leu Leu Lys Arg Asp Met Lys Ser Ala His Leu Leu Pro Glu  
290 295 300

His Ile Phe Asp Gly Glu Gly Leu Ala Ser Ser Phe Gly Tyr Asp Val  
305 310 315 320

Ala Val Met Asp Leu Asn Lys Asp Gly Trp Gln Asp Ile Val Ile Gly  
325 330 335

Ala Pro Gln Tyr Phe Asp Arg Asp Gly Glu Val Gly Gly Ala Val Tyr  
340 345 350

Val Tyr Met Asn Gln Gln Gly Arg Trp Asn Asn Val Lys Pro Ile Arg  
355 360 365

Leu Asn Gly Thr Lys Asp Ser Met Phe Gly Ile Ala Val Lys Asn Ile  
370 375 380

Gly Asp Ile Asn Gln Asp Gly Tyr Pro Asp Ile Ala Val Gly Ala Pro  
385 390 395 400

Tyr Asp Asp Leu Gly Lys Val Phe Ile Tyr His Gly Ser Ala Asn Gly  
405 410 415

Ile Asn Thr Lys Pro Thr Gln Val Leu Lys Gly Ile Ser Pro Tyr Phe  
420 425 430

Gly Tyr Ser Ile Ala Gly Asn Met Asp Leu Asp Arg Asn Ser Tyr Pro  
435 440 445

Asp Val Ala Val Gly Ser Leu Ser Asp Ser Val Thr Ile Phe Arg Ser  
450 455 460

Arg Pro Val Ile Asn Ile Gln Lys Thr Ile Thr Val Thr Pro Asn Arg  
465 470 475 480

Ile Asp Leu Arg Gln Lys Thr Ala Cys Gly Ala Pro Ser Gly Ile Cys  
485 490 495

Leu Gln Val Lys Ser Cys Phe Glu Tyr Thr Ala Asn Pro Ala Gly Tyr  
500 505 510

Asn Pro Ser Ile Ser Ile Val Gly Thr Leu Glu Ala Glu Lys Glu Arg  
515 520 525

Arg Lys Ser Gly Leu Ser Ser Arg Val Gln Phe Arg Asn Gln Gly Ser  
530 535 540

Glu Pro Lys Tyr Thr Gln Glu Leu Thr Leu Lys Arg Gln Lys Gln Lys  
545 550 555 560

Val Cys Met Glu Glu Thr Leu Trp Leu Gln Asp Asn Ile Arg Asp Lys  
565 570 575

Leu Arg Pro Ile Pro Ile Thr Ala Ser Val Glu Ile Gln Glu Pro Ser  
580 585 590

Ser Arg Arg Arg Val Asn Ser Leu Pro Glu Val Leu Pro Ile Leu Asn  
595 600 605

Ser Asp Glu Pro Lys Thr Ala His Ile Asp Val His Phe Leu Lys Glu  
610 615 620

Gly Cys Gly Asp Asp Asn Val Cys Asn Ser Asn Leu Lys Leu Glu Tyr  
625 630 635 640

Lys Phe Cys Thr Arg Glu Gly Asn Gln Asp Lys Phe Ser Tyr Leu Pro  
645 650 655

Ile Gln Lys Gly Val Pro Glu Leu Val Leu Lys Asp Gln Lys Asp Ile  
660 665 670

Ala Leu Glu Ile Thr Val Thr Asn Ser Pro Ser Asn Pro Arg Asn Pro  
675 680 685

Thr Lys Asp Gly Asp Asp Ala His Glu Ala Lys Leu Ile Ala Thr Phe  
690 695 700

Pro Asp Thr Leu Thr Tyr Ser Ala Tyr Arg Glu Leu Arg Ala Phe Pro  
705 710 715 720

Glu Lys Gln Leu Ser Cys Val Ala Asn Gln Asn Gly Ser Gln Ala Asp  
725 730 735

72

Cys Glu Leu Gly Asn Pro Phe Lys Arg Asn Ser Asn Val Thr Phe Tyr  
740 745 750

Leu Val Leu Ser Thr Thr Glu Val Thr Phe Asp Thr Pro Tyr Leu Asp  
755 760 765

Ile Asn Leu Lys Leu Glu Thr Thr Ser Asn Gln Asp Asn Leu Ala Pro  
770 775 780

Ile Thr Ala Lys Ala Lys Val Val Ile Glu Leu Leu Ser Val Ser  
785 790 795 800

Gly Val Ala Lys Pro Ser Gln Val Tyr Phe Gly Gly Thr Val Val Gly  
805 810 815

Glu Gln Ala Met Lys Ser Glu Asp Glu Val Gly Ser Leu Ile Glu Tyr  
820 825 830

Glu Phe Arg Val Ile Asn Leu Gly Lys Pro Leu Thr Asn Leu Gly Thr  
835 840 845

Ala Thr Leu Asn Ile Gln Trp Pro Lys Glu Ile Ser Asn Gly Lys Trp  
850 855 860

Leu Leu Tyr Leu Val Lys Val Glu Ser Lys Gly Leu Glu Lys Val Thr  
865 870 875 880

Cys Glu Pro Gln Lys Glu Ile Asn Ser Leu Asn Leu Thr Glu Ser His  
885 890 895

Asn Ser Arg Lys Lys Arg Glu Ile Thr Glu Lys Gln Ile Asp Asp Asn  
900 905 910

Arg Lys Phe Ser Leu Phe Ala Glu Arg Lys Tyr Gln Thr Leu Asn Cys  
915 920 925

Ser Val Asn Val Asn Cys Val Asn Ile Arg Cys Pro Leu Arg Gly Leu  
930 935 940

Asp Ser Lys Ala Ser Leu Ile Leu Arg Ser Arg Leu Trp Asn Ser Thr  
945 950 955 960

Phe Leu Glu Glu Tyr Ser Lys Leu Asn Tyr Leu Asp Ile Leu Met Arg  
965 970 975

Ala Phe Ile Asp Val Thr Ala Ala Glu Asn Ile Arg Leu Pro Asn  
980 985 990

Ala Gly Thr Gln Val Arg Val Thr Val Phe Pro Ser Lys Thr Val Ala  
995 1000 1005

73

Gln Tyr Ser Gly Val Pro Trp Trp Ile Ile Leu Val Ala Ile Leu Ala  
1010 1015 1020

Gly Ile Leu Met Leu Ala Leu Leu Val Phe Ile Leu Trp Lys Cys Gly  
1025 1030 1035 1040

Phe Phe Lys Arg Asn Lys Lys Asp His Tyr Asp Ala Thr Tyr His Lys  
1045 1050 1055

Ala Glu Ile His Ala Gln Pro Ser Asp Lys Glu Arg Leu Thr Ser Asp  
1060 1065 1070

Ala

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5629 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1..5629
- (D) OTHER INFORMATION: /product= "Human ALPHA 6A"  
/note= "SEQ ID NO: 2 is the 5629 base nucleotide sequence of the human ALPHA 6A cDNA."

(ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 147..149
- (D) OTHER INFORMATION: /function= "Transcription initiator"

(ix) FEATURE:

- (A) NAME/KEY: mat\_peptide
- (B) LOCATION: 216..3365
- (D) OTHER INFORMATION: /product= "Human ALPHA 6A"

(ix) FEATURE:

- (A) NAME/KEY: misc\_feature

(B) LOCATION: 3264..3278  
 (D) OTHER INFORMATION: /product= "The cytoplasmic sequence  
 GFFKR."

## (ix) FEATURE:

(A) NAME/KEY: misc\_feature  
 (B) LOCATION: 3261..3390  
 (D) OTHER INFORMATION: /note= "The 130 nucleotide sequence  
 present in SEQ ID NO: 2 but deleted from SEQ ID  
 NO:4."

## (ix) FEATURE:

(A) NAME/KEY: misc\_feature  
 (B) LOCATION: 2924..3455  
 (D) OTHER INFORMATION: /note= "The sequence of the ALPHA  
 6A cDNA amplified using primers 1156/1157."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

|                        |                                  |             |     |
|------------------------|----------------------------------|-------------|-----|
| GGCGGACCGT CCCGGGGGTG  | GGGCGGGCG CAGCGCGAG AGGAGGCGAA   | GGTGGCTGGC  | 60  |
| GTAGGAGCAG CGCGGCAGCC  | TGGACCCAG CCCGGAGCGC AGGGCGGCCG  | CTGCAGGTCC  | 120 |
| CCGCTCCCT CCCCGTGGT    | CCGCCCATGG CCGCCGCCGG GCAGCTGTGC | TTGCTCTACC  | 180 |
| TGTGGCGGG GCTGCTGTCC   | CGGCTCGCGC CAGCCTTCAA CTTGGACACT | CGGGAGGACA  | 240 |
| ACGTGATCCG GAAATATGGA  | GACCCCGGGA GCCTCTTCGG CTTCTCGCTG | GCCATGCACT  | 300 |
| GGCAACTGCA GCCCGAGGAC  | AAGCGGCTGT TGCTCGTGGG GGCCCCGCGC | GGAGAAGCGC  | 360 |
| TTCCACTGCA GAGAGCCTTC  | AGAACGGGAG GGCTGTACAG CTGCGACATC | ACCGCCCCGGG | 420 |
| GGCCATGCAC GCGGATCGAG  | TTTGATAACG ATGCTGACCC CACGTCAGAA | AGCAAGGAAG  | 480 |
| ATCAGTGGAT GGGGGTCACC  | GTCCAGAGCC AAGGTCCAGG GGGCAAGGTC | GTGACATGTG  | 540 |
| CTCACCGATA TGAAAAAAAGG | CACCATGTTA ATACGAAGCA GGAATCCGA  | GACATCTTG   | 600 |
| GGCGGTGTTA TGTCTGAGT   | CAGAATCTCA GGATTGAAGA CGATATGGAT | GGGGGAGATT  | 660 |
| GGAGCTTTG TGATGGCGA    | TTGAGAGGCC ATGAGAAATT TGGCTCTTGC | CAGCAAGGTG  | 720 |
| TAGCAGCTAC TTTTACTAAA  | GACTTCATT ACATTGTATT TGGAGCCCCG  | GGTACTTATA  | 780 |
| ACTGGAAAGG GATTGTTCGT  | GTAGAGCAA AGAATAACAC TTTTTTGAC   | ATGAACATCT  | 840 |
| TTGAAGATGG GCCTTATGAA  | GTTGGTGGAG AGACTGAGCA TGATGAAAGT | CTCGTTCCCTG | 900 |
| TTCCTGCTAA CAGTTACTTA  | GGTTTTCTT TGGACTCAGG GAAAGGTATT  | GTTTCTAAAG  | 960 |

|  |      |
|--|------|
| ATGAGATCAC TTTGTATCT GGTGCTCCA GAGCCAATCA CAGTGGAGCC GTGGTTTGC     | 1020 |
| TGAAGAGAGA CATGAAGTCT GCACATCTCC TCCCTGAGCA CATATTCGAT GGAGAAGGTC  | 1080 |
| TGGCCTCTTC ATTTGGCTAT GATGTGGCGG TGATGGACCT CAACAAGGAT GGGTGGCAAG  | 1140 |
| ATATACTTAT TGGAGCCCCA CAGTATTTG ATAGAGATGG AGAAGTTGGA GGTGCAGTGT   | 1200 |
| ATGCTACAT GAACCAGCAA GGCAGATGGA ATAATGTGAA GCCAATTGGT CTTAATGGAA   | 1260 |
| CCAAAGATTC TATGTTGGC ATTGCAGTAA AAAATATTGG AGATATTAAT CAAGATGGCT   | 1320 |
| ACCCAGATAT TGCAGTTGGA GCTCCGTATG ATGACTTGGG AAAGGTTTT ATCTATCATG   | 1380 |
| GATCTGAAA TGGAATAAAAT ACCAAACCAA CACAGGTTCT CAAGGGTATA TCACCTTATT  | 1440 |
| TTGGATATTG AATTGCTGGA AACATGGACC TTGATCGAAA TTCTACCCCT GATGTTGCTG  | 1500 |
| TTGGTTCCCT CTCAGATTCA GTAACTATTT TCAGATCCCG CCCTGTGATT AATATTCAAGA | 1560 |
| AAACCATCAC AGTAACTCCT AACAGAATTG ACCTCCGCCA GAAAACAGCG TGTGGGGCGC  | 1620 |
| CTAGTGGGAT ATGCCTCCAG GTTAAATCCT GTTTGAATA TACTGCTAAC CCCGCTGGTT   | 1680 |
| ATAATCCTTC AATATCAATT GTGGGCACAC TTGAAGCTGA AAAAGAAAGA AGAAAATCTG  | 1740 |
| GGCTATCCTC AAGAGTTCAAG TTTGAAACC AAGGTTCTGA GCCCAAATAT ACTCAAGAAC  | 1800 |
| TAACTCTGAA GAGGCAGAAA CAGAAAGTGT GCATGGAGGA AACCCCTGTGG CTACAGGATA | 1860 |
| ATATCAGAGA TAAACTGCGT CCCATTGCCA TAACTGCCCTC AGTGGAGATC CAAGAGCCAA | 1920 |
| GCTCTCGTAG GCGAGTGAAT TCACTTCCAG AAGTTCTTCC AATTCTGAAT TCAGATGAAC  | 1980 |
| CCAAGACAGC TCATATTGAT GTTCACTTCT TAAAAGAGGG ATGTGGAGAC GACAATGTAT  | 2040 |
| GTAACAGCAA CCTTAAACTA GAATATAAAAT TTTGCACCCG AGAAGGAAAT CAAGACAAAT | 2100 |
| TTTCTTATTT ACCAATTCAA AAAGGTGTAC CAGAACTAGT TCTAAAAGAT CAGAAGGATA  | 2160 |
| TTGCTTTAGA AATAACAGTG ACAAACAGCC CTTCCAACCC AAGGAATCCC ACAAAAGATG  | 2220 |
| GCGATGACGG CCATGAGGCT AAACTGATTG CAACGTTCC AGACACTTTA ACCTATTCTG   | 2280 |
| CATATAGAGA ACTGAGGGCT TTCCCTGAGA AACAGTTGAG TTGTGTTGCC AACCGAGATG  | 2340 |
| GCTCGCAAGC TGACTGTGAG CTGGAAATC CTTTAAAG AAATTCAAAT GTCACTTTT      | 2400 |
| ATTTGGTTTT AAGTACAACG GAAGTCACCT TTGACACCCCA ATATCTGGAT ATTAATCTGA | 2460 |
| AGTTAGAAAC ACAAGCAAT CAAGATAATT TGGCTCCAAT TACAGCTAAA GCAAAAGTGG   | 2520 |

|  |      |
|--|------|
| TTATTGAACT GCTTTATCG GTCTGGGAG TTGCTAAACC TTCCCAGGTG TATTTGGAG     | 2580 |
| GTACAGTTGT TGGCGAGCAA CCTATGAAAT CTGAAGATGA ACTGGGAAGT TTAATAGAGT  | 2640 |
| ATGAATTCAAG GGTAAATAAAC TTAGGTAAAC CTCTTACAAA CCTGGCACA GCAACCTTGA | 2700 |
| ACATTCACTG GCCAAAAGAA ATTAGCAATG GGAAATGGTT GCTTTATTG GTGAAAGTAG   | 2760 |
| AATCCAAAGG ATTGGAAAAG GTAACTTGTG AGCCACAAAA GGAGATAAAC TCCCTGAACC  | 2820 |
| TAACGGAGTC TCACAACCTCA AGAAAGAAC GGGAAATTAC TGAAAAACAG ATAGATGATA  | 2880 |
| ACAGAAAATT TTCTTATTG GCTGAAAGAA AATACCAGAC TCTTAACGT AGCGTGAACG    | 2940 |
| TGAACTGTGT GAACATCAGA TGCCCGCTGC GGGGGCTGGA CAGCAAGGCG TCTCTTA     | 3000 |
| TGCGCTCGAG GTTATGGAAC AGCACATTG TAGAGGAATA TTCCAAACTG AACTACTTGG   | 3060 |
| ACATTCTCAT GCGAGCCTTC ATTGATGTGA CTGCTGCTGC CGAAAATATC AGGCTGCCAA  | 3120 |
| ATGCAGGCAC TCAGGTTCGA GTGACTGTGT TTCCCTCAAA GACTGTAGCT CAGTATTGG   | 3180 |
| GAGTACCTTG GTGGATCATC CTAGTGGCTA TTCTCGCTGG GATCTTGATG CTTGCTTTAT  | 3240 |
| TAGTGTAT ACTATGGAAG TGTGGTTCT TCAAGAGAAA TAAGAAAGAT CATTATGATG     | 3300 |
| CCACATATCA CAAGGCTGAG ATCCATGCTC AGCCATCTGA TAAAGAGAGG CTTACTTCTG  | 3360 |
| ATGCATAGTA TTGATCTACT TCTGTAATTG TGTGGATTCT TTAAACGCTC TAGGTACGAT  | 3420 |
| GACAGTGTTC CCCGATACCA TGCTGTAAGG ATCCGGAAAG AAGAGCGAGA GATCAAAGAT  | 3480 |
| GAAAAGTATA TTGATAACCT TGAAAAAAA CAGTGGATCA CAAAGTGGAA CAGAAATGAA   | 3540 |
| AGCTACTCAT AGCGGGGGCC TAAAAAAA AAAGCTTCAC AGTACCCAAA CTGCTTTTC     | 3600 |
| CAAATCAGAA ATTCAATTG GATTAAAAG CCTGCTCAAT CCCTGAGGAC TGATTCAGA     | 3660 |
| GTGACTACAC ACAGTACGAA CCTACAGTT TAACTGTGGA TATTGTTACG TAGCCTAAGG   | 3720 |
| CTCGTGTGTT GCACAGCCAA ATTAAAAGT GTTGGAAATGG ATTTTCTTT AACTGCCGTA   | 3780 |
| ATTTAACTTT CTGGGTTGCC TTTGTTTTG GCGTGGCTGA CTTACATCAT GTGTTGGGA    | 3840 |
| AGGGCCTGCC CAGTTGCACT CAGGTGACAT CCTCCAGATA GTGTAGCTGA GGAGGCACCT  | 3900 |
| ACACTCACCT GCACTAACAG AGTGGCCGTC CTAACCTCGG CCCTGCTGCG CAGACGTCCA  | 3960 |
| TCACGTTAGC TGTCCCACAT CACAAGACTA TGCCATTGGG GTAGTTGTGT TTCAACGGAA  | 4020 |
| AGTGCTGTCT TAAACTAAAT GTGCAATAGA AGGTGATGTT GCCATCCTAC CGTCTTTCC   | 4080 |

|            |            |             |            |            |            |      |
|------------|------------|-------------|------------|------------|------------|------|
| TGTTTCCTAG | CTGTGTGAAT | ACCTGCTCAC  | GTCAAATGCA | TACAAGTTTC | ATTCTCCCTT | 4140 |
| TCACTAAAAA | CACACAGGTG | CAACAGACTT  | GAATGCTAGT | TATACTTATT | TGTATATGGT | 4200 |
| ATTTATTTT  | TCTTTCTTT  | ACAAACCATT  | TTGTTATTGA | CTAACAGGCC | AAAGAGTCTC | 4260 |
| CAGTTACCC  | TTCAGGTTGG | TTAACATCAAT | CAGAATTAGA | ATTAGAGCAT | GGGAGGGTCA | 4320 |
| TCACTATGAC | CTAAATTATT | TACTGCAAAA  | AGAAAATCTT | TATAAATGTA | CCAGAGAGAG | 4380 |
| TTGTTTAAT  | AACTTATCTA | TAAACTATAA  | CCTCTCCTTC | ATGACAGCCT | CCACCCACA  | 4440 |
| ACCCAAAAGG | TTAAGAAAAT | AGAATTATAA  | CTGTAAAGAT | GTTCATTTCA | GGCATTGGAT | 4500 |
| ATTTTTACT  | TTAGAAGCCT | GCATAATGTT  | TCTGGATTAA | CATACTGTA  | CATTAGGAA  | 4560 |
| TTCTGGAGA  | AGATGGGTTT | ATTCACTGAA  | CTCTAGTGC  | GTTTACTCAC | TGCTGCAAAT | 4620 |
| ACTGTATATT | CAGGACTTGA | AAGAAATGGT  | GAATGCCAT  | GGAACTAGTG | GATCCAAACT | 4680 |
| GATCCAGTAT | AAGACTACTG | AATCTGCTAC  | CAAACAGTT  | AATCACTGAG | TCGAGTGTTC | 4740 |
| TATTTTTGT  | TTGTTTCCT  | CCCCTATCTG  | TATTCCAAA  | AATTACTTG  | GGGCTAATTT | 4800 |
| AACAAGAACT | TTAAATTGTG | TTTAATTGT   | AAAAATGGCA | GGGGGTGGAA | TTATTACTCT | 4860 |
| ATACATTCAA | CAGAGACTGA | ATAGATATGA  | AAGCTGATT  | TTTTTAATT  | CCATGCTTCA | 4920 |
| CAATGTTAAG | TTATATGGGG | AGCAACAGCA  | AACAGGTGCT | AATTGTTTT  | GGATATACTA | 4980 |
| TAAGCAGTGT | CTGTGTTTG  | AAAGAATAGA  | ACACAGTTG  | TAGTGCCACT | GTTGTTTG   | 5040 |
| GGGGGGCTTT | TTTCTTTTT  | CCGGAAAATC  | CTTAAACCTT | AAGATACTAA | GGACGTTGTT | 5100 |
| TTGGTTGTAC | TTGGAATTCT | TAGTCACAAA  | ATATATTG   | TTTACAAAAA | TTTCTGTAAA | 5160 |
| ACAGGTTATA | ACAGTGTAA  | AACTCTCAGT  | TTCTGCTTG  | GGGAACTTGT | GTCCCTAATG | 5220 |
| TGTTAGATTG | CTAGATTGCT | AAGGAGCTGA  | TACTGACAG  | TTTTTAGAC  | CTGTGTTACT | 5280 |
| AAAAAAAAGA | TGAATGTCGG | AAAAGGGTGT  | TGGGAGGGTG | GTCAACAAAG | AAACAAAGAT | 5340 |
| GTTATGGTGT | TTAGACTTAT | GGTTGTTAAA  | AATGTCATCT | CAAGTCAGT  | CACTGGTCTG | 5400 |
| TTTGCATTTG | ATACATTGTT | GTACTAACTA  | GCATTGTA   | ATTATTCAT  | GATTAGAAAT | 5460 |
| TACCTGTGGA | TATTTGTATA | AAAGTGTGAA  | ATAAATTGTT | TATAAAAGTG | TTCATTGTT  | 5520 |
| CGTAACACAG | CATTGTATAT | GTGAAGCAA   | CTCTAAAATT | ATAAATGACA | ACCTGAATT  | 5580 |
| TCTATTCAT  | CAAAAAAAA  | AAAAAAA     | ACTTTATGGG | CACAACTGG  |            | 5629 |

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1091 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..1091
- (D) OTHER INFORMATION: /note= "SEQ ID NO:3 is the 1091 residue amino acid sequence of the human ALPHA 6B protein."

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..1044
- (D) OTHER INFORMATION: /note= "The sequence of SEQ ID NO:3 is identical to SEQ ID NO:1 between amino acids 1 and 1044."

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 927..1060
- (D) OTHER INFORMATION: /note= "Encompasses the sequence encoded by the fragment of ALPHA 6B cDNA amplified using primers 1156/1157."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Ala Ala Ala Gly Gln Leu Cys Leu Leu Tyr Leu Ser Ala Gly Leu  
1 5 10 15

Leu Ser Arg Leu Gly Ala Ala Phe Asn Leu Asp Thr Arg Glu Asp Asn  
20 25 30

Val Ile Arg Lys Tyr Gly Asp Pro Gly Ser Leu Phe Gly Phe Ser Leu  
35 40 45

Ala Met His Trp Gln Leu Gln Pro Glu Asp Lys Arg Leu Leu Leu Val  
50 55 60

79

Gly Ala Pro Arg Gly Glu Ala Leu Pro Leu Gln Arg Ala Phe Arg Thr  
65 70 75 80

Gly Gly Leu Tyr Ser Cys Asp Ile Thr Ala Arg Gly Pro Cys Thr Arg  
85 90 95

Ile Glu Phe Asp Asn Asp Ala Asp Pro Thr Ser Glu Ser Lys Glu Asp  
100 105 110

Gln Trp Met Gly Val Thr Val Gln Ser Gln Gly Pro Gly Gly Lys Val  
115 120 125

Val Thr Cys Ala His Arg Tyr Glu Lys Arg Gln His Val Asn Thr Lys  
130 135 140

Gln Glu Ser Arg Asp Ile Phe Gly Arg Cys Tyr Val Leu Ser Gln Asn  
145 150 155 160

Leu Arg Ile Glu Asp Asp Met Asp Gly Gly Asp Trp Ser Phe Cys Asp  
165 170 175

Gly Arg Leu Arg Gly His Glu Lys Phe Gly Ser Cys Gln Gln Gly Val  
180 185 190

Ala Ala Thr Phe Thr Lys Asp Phe His Tyr Ile Val Phe Gly Ala Pro  
195 200 205

Gly Thr Tyr Asn Trp Lys Gly Ile Val Arg Val Glu Gln Lys Asn Asn  
210 215 220

Thr Phe Phe Asp Met Asn Ile Phe Glu Asp Gly Pro Tyr Glu Val Gly  
225 230 235 240

Gly Glu Thr Glu His Asp Glu Ser Leu Val Pro Val Pro Ala Asn Ser  
245 250 255

Tyr Leu Gly Phe Ser Leu Asp Ser Gly Lys Gly Ile Val Ser Lys Asp  
260 265 270

Glu Ile Thr Phe Val Ser Gly Ala Pro Arg Ala Asn His Ser Gly Ala  
275 280 285

Val Val Leu Leu Lys Arg Asp Met Lys Ser Ala His Leu Leu Pro Glu  
290 295 300

His Ile Phe Asp Gly Glu Gly Leu Ala Ser Ser Phe Gly Tyr Asp Val  
305 310 315 320

Ala Val Met Asp Leu Asn Lys Asp Gly Trp Gln Asp Ile Val Ile Gly  
325 330 335

80

Ala Pro Gln Tyr Phe Asp Arg Asp Gly Glu Val Gly Ala Val Tyr  
340 345 350

Val Tyr Met Asn Gln Gln Gly Arg Trp Asn Asn Val Lys Pro Ile Arg  
355 360 365

Leu Asn Gly Thr Lys Asp Ser Met Phe Gly Ile Ala Val Lys Asn Ile  
370 375 380

Gly Asp Ile Asn Gln Asp Gly Tyr Pro Asp Ile Ala Val Gly Ala Pro  
385 390 395 400

Tyr Asp Asp Leu Gly Lys Val Phe Ile Tyr His Gly Ser Ala Asn Gly  
405 410 415

Ile Asn Thr Lys Pro Thr Gln Val Leu Lys Gly Ile Ser Pro Tyr Phe  
420 425 430

Gly Tyr Ser Ile Ala Gly Asn Met Asp Leu Asp Arg Asn Ser Tyr Pro  
435 440 445

Asp Val Ala Val Gly Ser Leu Ser Asp Ser Val Thr Ile Phe Arg Ser  
450 455 460

Arg Pro Val Ile Asn Ile Gln Lys Thr Ile Thr Val Thr Pro Asn Arg  
465 470 475 480

Ile Asp Leu Arg Gln Lys Thr Ala Cys Gly Ala Pro Ser Gly Ile Cys  
485 490 495

Leu Gln Val Lys Ser Cys Phe Glu Tyr Thr Ala Asn Pro Ala Gly Tyr  
500 505 510

Asn Pro Ser Ile Ser Ile Val Gly Thr Leu Glu Ala Glu Lys Glu Arg  
515 520 525

Arg Lys Ser Gly Leu Ser Ser Arg Val Gln Phe Arg Asn Gln Gly Ser  
530 535 540

Glu Pro Lys Tyr Thr Gln Glu Leu Thr Leu Lys Arg Gln Lys Gln Lys  
545 550 555 560

Val Cys Met Glu Glu Thr Leu Trp Leu Gln Asp Asn Ile Arg Asp Lys  
565 570 575

Leu Arg Pro Ile Pro Ile Thr Ala Ser Val Glu Ile Gln Glu Pro Ser  
580 585 590

Ser Arg Arg Arg Val Asn Ser Leu Pro Glu Val Leu Pro Ile Leu Asn  
595 600 605

81

Ser Asp Glu Pro Lys Thr Ala His Ile Asp Val His Phe Leu Lys Glu  
610 615 620

Gly Cys Gly Asp Asp Asn Val Cys Asn Ser Asn Leu Lys Leu Glu Tyr  
625 630 635 640

Lys Phe Cys Thr Arg Glu Gly Asn Gln Asp Lys Phe Ser Tyr Leu Pro  
645 650 655

Ile Gln Lys Gly Val Pro Glu Leu Val Leu Lys Asp Gln Lys Asp Ile  
660 665 670

Ala Leu Glu Ile Thr Val Thr Asn Ser Pro Ser Asn Pro Arg Asn Pro  
675 680 685

Thr Lys Asp Gly Asp Asp Ala His Glu Ala Lys Leu Ile Ala Thr Phe  
690 695 700

Pro Asp Thr Leu Thr Tyr Ser Ala Tyr Arg Glu Leu Arg Ala Phe Pro  
705 710 715 720

Glu Lys Gln Leu Ser Cys Val Ala Asn Gln Asn Gly Ser Gln Ala Asp  
725 730 735

Cys Glu Leu Gly Asn Pro Phe Lys Arg Asn Ser Asn Val Thr Phe Tyr  
740 745 750

Leu Val Leu Ser Thr Thr Glu Val Thr Phe Asp Thr Pro Tyr Leu Asp  
755 760 765

Ile Asn Leu Lys Leu Glu Thr Thr Ser Asn Gln Asp Asn Leu Ala Pro  
770 775 780

Ile Thr Ala Lys Ala Lys Val Val Ile Glu Leu Leu Ser Val Ser  
785 790 795 800

Gly Val Ala Lys Pro Ser Gln Val Tyr Phe Gly Gly Thr Val Val Gly  
805 810 815

Glu Gln Ala Met Lys Ser Glu Asp Glu Val Gly Ser Leu Ile Glu Tyr  
820 825 830

Glu Phe Arg Val Ile Asn Leu Gly Lys Pro Leu Thr Asn Leu Gly Thr  
835 840 845

Ala Thr Leu Asn Ile Gln Trp Pro Lys Glu Ile Ser Asn Gly Lys Trp  
850 855 860

Leu Leu Tyr Leu Val Lys Val Glu Ser Lys Gly Leu Glu Lys Val Thr  
865 870 875 880

Cys Glu Pro Gln Lys Glu Ile Asn Ser Leu Asn Leu Thr Glu Ser His  
 885 890 895  
 Asn Ser Arg Lys Lys Arg Glu Ile Thr Glu Lys Gln Ile Asp Asp Asn  
 900 905 910  
 Arg Lys Phe Ser Leu Phe Ala Glu Arg Lys Tyr Gln Thr Leu Asn Cys  
 915 920 925  
 Ser Val Asn Val Asn Cys Val Asn Ile Arg Cys Pro Leu Arg Gly Leu  
 930 935 940  
 Asp Ser Lys Ala Ser Leu Ile Leu Arg Ser Arg Leu Trp Asn Ser Thr  
 945 950 955 960  
 Phe Leu Glu Glu Tyr Ser Lys Leu Asn Tyr Leu Asp Ile Leu Met Arg  
 965 970 975  
 Ala Phe Ile Asp Val Thr Ala Ala Ala Glu Asn Ile Arg Leu Pro Asn  
 980 985 990  
 Ala Gly Thr Gln Val Arg Val Thr Val Phe Pro Ser Lys Thr Val Ala  
 995 1000 1005  
 Gln Tyr Ser Gly Val Pro Trp Trp Ile Ile Leu Val Ala Ile Leu Ala  
 1010 1015 1020  
 Gly Ile Leu Met Leu Ala Leu Val Phe Ile Leu Trp Lys Cys Gly  
 1025 1030 1035 1040  
 Phe Phe Lys Arg Ser Arg Tyr Asp Asp Ser Val Pro Arg Tyr His Ala  
 1045 1050 1055  
 Val Arg Ile Arg Lys Glu Glu Arg Glu Ile Lys Asp Glu Lys Tyr Ile  
 1060 1065 1070  
 Asp Asn Leu Glu Lys Lys Gln Trp Ile Thr Lys Trp Asn Arg Asn Glu  
 1075 1080 1085  
 Ser Tyr Ser  
 1090

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 5499 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(ix) FEATURE:

(A) NAME/KEY: misc\_feature

(B) LOCATION: 1..5499

(D) OTHER INFORMATION: /product= "Human ALPHA 6B"

(ix) FEATURE:

(A) NAME/KEY: misc\_feature

(B) LOCATION: 1..3260

(D) OTHER INFORMATION: /note= "The sequence of SEQ ID NO:4 is identical to SEQ ID NO:2 between nucleotides 1 and 3260."

(ix) FEATURE:

(A) NAME/KEY: misc\_feature

(B) LOCATION: 3261..5499

(D) OTHER INFORMATION: /note= "Nucleotides 3261-5499 of SEQ ID NO:4 are identical to nucleotides 3391-5629 of SEQ ID NO:2. SEQ ID NO:4 has a 130 nucleotide deletion in relation to SEQ ID NO:2."

(ix) FEATURE:

(A) NAME/KEY: misc\_feature

(B) LOCATION: 2924..3325

(D) OTHER INFORMATION: /note= "Encompasses the sequence of the ALPHA 6B cDNA amplified using primers 1156/1157."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

|   |     |
|---|-----|
| GCGCGACCGT CCCGGGGGTG GGGCCGGCG CAGCGGCGAG AGGAGGCGAA GGTGGCTGCG  | 60  |
| GTAGCAGCAG CGCGGCAGCC TCGGACCCAG CCCGGAGCGC AGGGCGGGCG CTGCAGGTCC | 120 |
| CCGCTCCGCT CCCCGTGCCT CCGCCCATGG CCGCCGCCGG GCAGCTGTGC TTGCTCTACC | 180 |
| TGTGGGGGG GCTCCTGTCC CGGCTGGGG CAGCCTTCAA CTTGGACACT CGGGAGGACA   | 240 |
| ACGTGATCCG GAAATATGGA GACCCGGGA GCCTCTTCGG CTTCTCGCTG GCCATGCACT  | 300 |
| GGCAACTGCA GCCCGAGGAC AAGGGCTGT TGCTCGTGGG GGCCCCGCGC GGAGAAGCGC  | 360 |
| TTCCACTGCA GAGAGCCTTC AGAACGGAG GGCTGTACAG CTGCGACATC ACCGCCCCGG  | 420 |
| GGCCATGCAC GCGGATCGAG TTTGATAACG ATGCTGACCC CACGTGAGAA AGCAAGGAAG | 480 |

|  |      |
|--|------|
| ATCAGTGGAT GGGGGTCACC GTCCAGAGCC AAGGTCCAGG GGGCAAGGTC GTGACATGTG  | 540  |
| CTCACCGATA TGAAAAAAGG CAGCATGTTA ATACGAAGCA GGAATCCCGA GACATCTTG   | 600  |
| GGCGGTGTTA TGTCTGAGT CAGAATCTCA GGATTGAAGA CGATATGGAT GGGGGAGATT   | 660  |
| GGAGCTTTG TGATGGCGA TTGAGAGGCC ATGAGAAATT TGGCTCTG CAGCAAGGTG      | 720  |
| TAGCAGCTAC TTTTACTAAA GACTTCATT ACATTGTATT TGGAGCCCCG GGTACTTATA   | 780  |
| ACTGGAAAGG GATTGTTCGT GTAGAGCAAA AGAATAACAC TTTTTTGAC ATGAACATCT   | 840  |
| TTGAAGATGG GCCTTATGAA GTTGGTGGAG AGACTGAGCA TGATGAAAGT CTCGTTCTG   | 900  |
| TTCCTGCTAA CAGTTACTTA GGTTTTCTT TGGACTCAGG GAAAGGTATT GTTTCTAAAG   | 960  |
| ATGAGATCAC TTTTGTATCT GGTGCTCCCA GAGCCAATCA CAGTGGAGCC GTGGTTTG    | 1020 |
| TGAAGAGAGA CATGAAGTCT GCACATCTCC TCCCTGAGCA CATATTGAT GGAGAAGGTG   | 1080 |
| TGGCCTCTTC ATTTGGCTAT GATGTGGCGG TGATGGACCT CAACAAGGAT GGGTGGCAAG  | 1140 |
| ATATAGTTAT TGGAGCCCCA CAGTATTTG ATAGAGATGG AGAAGTTGGA GGTGCAGTGT   | 1200 |
| ATGTCTACAT GAACCAGCAA GGCAGATGGA ATAATGTGAA GCCAATTGCT CTTAATGGAA  | 1260 |
| CCAAAGATTG TATGTTGGC ATTGCAGTAA AAAATATTGG AGATATTAAT CAAAGATGGCT  | 1320 |
| ACCCAGATAT TGCAGTTGGA GCTCCGTATG ATGACTTGGG AAAGGTTTT ATCTATCATG   | 1380 |
| GATCTGCAAA TGGAATAAAAT ACCAAACCAA CACAGGTTCT CAAGGGTATA TCACCTTATT | 1440 |
| TTGGATATTG AATTGCTGGA AACATGGACC TTGATCGAAA TTCCCTACCT GATGTTGCTG  | 1500 |
| TTGGTTCCCT CTCAGATTCA GTAACTATTG TCAGATCCCG GCCTGTGATT AATATTGAGA  | 1560 |
| AAACCATCAC AGTAACCTCCT AACAGAATTG ACCTCCGCCA GAAAACAGCG TGTGGGGCGC | 1620 |
| CTAGTGGAT ATGCCTCCAG GTTAAATCCT GTTTGAATA TACTGCTAAC CCGCTGGTT     | 1680 |
| ATAATCCTTC AATATCAATT GTGGGCACAC TTGAAGCTGA AAAAGAAAGA AGAAAATCTG  | 1740 |
| GGCTATCCTC AAGAGTTCAAG TTTCGAAACC AAGGTTCTGA GCCCAAATAT ACTCAAGAAC | 1800 |
| TAACTGTGAA GAGGCAGAAA CAGAAAGTGT GCATGGAGGA AACCTGTGG CTACAGGATA   | 1860 |
| ATATCAGAGA TAAACTGCGT CCCATTCCCA TAACTGCCTC AGTGGAGATC CAAGAGCCAA  | 1920 |
| GCTCTCGTAG GCGAGTGAAT TCACTTCCAG AAGTTCTTCC AATTCTGAAT TCAGATGAAC  | 1980 |
| CCAAGACAGC TCATATTGAT GTTCACCTCT TAAAAGAGGG ATGTGGAGAC GACAATGTAT  | 2040 |

|  |      |
|--|------|
| GTAACAGCAA CCTTAAACTA GAATATAAAT TTTGCACCCG AGAAGGAAAT CAAGACAAAT  | 2100 |
| TTTCTTATTT ACCAATTCAA AAAGGTGTAC CAGAACTAGT TCTAAAAGAT CAGAAGGATA  | 2160 |
| TTGCTTACA AATAACAGTG ACAAAACAGCC CTTCCAACCC AAGGAATCCC ACAAAAGATG  | 2220 |
| GCGATGACGC CCATGAGGCT AAACGTGATTG CAACGTTCC AGACACTTTA ACCTATTCTG  | 2280 |
| CATATAGAGA ACTGAGGGCT TTCCCTGAGA AACAGTTGAG TTGTGTTGCC AACCGAGAATG | 2340 |
| GCTCGCAAGC TGACTGTGAG CTCGGAAATC CTTTTAAAAG AAATTCAAAT GTCACTTTT   | 2400 |
| ATTTGGTTTT AAGTACAACG GAAGTCACCT TTGACACCCC ATATCTGGAT ATTAATCTGA  | 2460 |
| AGTTAGAAC AACAAAGCAAT CAAGATAATT TGGCTCCAAT TACAGCTAAA GCAAAAGTGG  | 2520 |
| TTATTGAACT GCTTTATCG GTCTCGGGAG TTGCTAAACC TTCCCAGGTG TATTTGGAG    | 2580 |
| GTACAGTTGT TGGCGAGCAA GCTATGAAAT CTGAAGATGA AGTGGGAAGT TTAATAGAGT  | 2640 |
| ATGAATTGAG GGTAATAAAC TTAGGTAAAC CTCTTACAAA CCTCGGCACA GCAACCTTGA  | 2700 |
| ACATTCACTG GCCAAAAGAA ATTAGCAATG GGAAATGGTT GCTTTATTG GTGAAAGTAG   | 2760 |
| AATCCAAAGG ATTGGAAAAG GTAACTTGTG AGCCACAAAA GGAGATAAAAC TCCCTGAACC | 2820 |
| TAACGGAGTC TCACAACTCA AGAAAGAAC GGGAAATTAC TGAAAAACAG ATAGATGATA   | 2880 |
| ACAGAAAATT TTCTTATTT GCTGAAAGAA AATACCAGAC TCTTAACGT AGCGTGAACG    | 2940 |
| TGAACGTGT GAACATCAGA TGCCCCCTGC GGGGGCTGGA CAGCAAGGCG TCTCTTATT    | 3000 |
| TGCGCTCGAG GTTATGGAAC ACCACATTG TAGAGGAATA TTCCAAACTG AACTACTTGG   | 3060 |
| ACATTCTCAT GCGAGCCTTC ATTGATGTGA CTGCTGCTGC CGAAAATATC AGGCTGCCAA  | 3120 |
| ATGCAGGCAC TCAGGTTCGA GTGACTGTGT TTCCCTCAAA GACTGTAGCT CAGTATTGG   | 3180 |
| GAGTACCTTG GTGGATCATC CTAGTGGCTA TTCTCGCTGG GATCTTGATG CTTCGTTAT   | 3240 |
| TAGTGTATTACTATGGAAG TGTGGATTCT TTAAACGCTC TAGGTACGGAT GACAGTGTTC   | 3300 |
| CCCGATACCA TGCTGTAAGG ATCCGGAAAG AAGAGCGAGA GATCAAAGAT GAAAAGTATA  | 3360 |
| TTGATAACCT TGAAAAAAA CAGTGGATCA CAAAGTGGAA CAGAAATGAA AGCTACTCAT   | 3420 |
| AGCGGGGGCC TAAAAAAA AAAGCTTCAC AGTACCCAAA CTGCTTTTC CAACTCAGAA     | 3480 |
| ATTCAATTG GATTAAAAAG CCTGCTCAAT CCCTGAGGAC TGATTCAGA GTGACTACAC    | 3540 |
| ACAGTACGAA CCTACAGTTT TAACTGTGGA TATTGTTACG TAGCCTAAGG CTCCTGTTT   | 3600 |

|  |      |
|--|------|
| CCACAGCCAA ATTTAAAACG GTTGAATGG ATTTTCTT AACTGCCGTA ATTTAACCTT     | 3660 |
| CTGGGTTGCC TTTGTTTTG GCGTGGCTGA CTTACATCAT GTGTTGGGA AGGGCCTGCC    | 3720 |
| CAGTTGCACT CAGGTGACAT CCTCCAGATA GTGTAGCTGA GGAGGCACCT ACACTCACCT  | 3780 |
| GCACTAACAG AGTGGCCGTC CTAACCTCGG GCCTGCTGCG CAGACGTCCA TCACGTTAGC  | 3840 |
| TGTCCCACAT CACAAGACTA TGCCATTGGG GTAGTTGTGT TTCAACGGAA AGTGCTGTCT  | 3900 |
| TAAACTAAAT GTGCAATAGA AGGTGATGTT GCCATCCTAC CGTCTTTCC TGTTTCCTAG   | 3960 |
| CTGTGTGAAT ACCTGCTCAC GTCAAATGCA TACAAGTTTC ATTCTCCCTT TCACTAAAAA  | 4020 |
| CACACAGGTG CAACAGACTT GAATGCTAGT TATACTTATT TGTATATGGT ATTTATTTTT  | 4080 |
| TCTTTCTTT ACAAAACCATT TTGTTATTGA CTAACAGGCC AAAGAGTCTC CAGTTACCC   | 4140 |
| TTCAGGTTGG TTTAATCAAT CAGAATTAGA ATTAGAGCAT GGGAGGGTCA TCACTATGAC  | 4200 |
| CTAAATTATT TACTGCAAAA AGAAAATCTT TATAATGTA CCAGAGAGAG TTGTTTTAAT   | 4260 |
| AACTTATCTA TAAACTATAA CCTCTCCTTC ATGACAGCCT CCACCCACCA ACCCAAAAGG  | 4320 |
| TTTAAGAAAT AGAATTATAA CTGTAAAGAT GTTTATTCA GGCATTGGAT ATTTTTACT    | 4380 |
| TTAGAACCT GCATAATGTT TCTGGATTAA CATACTGTAA CATTCAAGGAA TTCTGGAGA   | 4440 |
| AGATGGGTTT ATTCACTGAA CTCTAGTGCG GTTTACTCAC TGCTGCAAAT ACTGTATATT  | 4500 |
| CAGGACTTGA AAGAAATGGT GAATGCCTAT GGAACCTAGTG GATCCAAACT GATCCAGTAT | 4560 |
| AAGACTACTG AATCTGCTAC CAAACAGTT AATCAGTGAG TCGAGTGTTC TATTTTTGT    | 4620 |
| TTTGTTCCT CCCCTATCTG TATTCCAAA AATTACTTTG GGGCTAATTT ACAACAAACT    | 4680 |
| TTAAATTGTG TTTAATTGT AAAAATGGCA GGGGGTGGAA TTATTACTCT ATACATTCAA   | 4740 |
| CAGAGACTGA ATAGATATGA AAGCTGATTT TTTTAATTA CCATGCTTCA CAATGTTAAG   | 4800 |
| TTATATGGGG AGCAACAGCA AACAGGTGCT AATTGTTTT GGATATAGTA TAAGCAGTGT   | 4860 |
| CTGTGTTTG AAAGAAATAGA ACACAGTTG TAGTGCCACT CTTGTTTG GGGGGGCTTT     | 4920 |
| TTTTCTTTT CCGGAAAATC CTTAACCTT AAGATACTAA GGACGTTGTT TTGGTTGTAC    | 4980 |
| TTGGAATTCT TAGTCACAAA ATATATTTG TTTACAAAAA TTTCTGTAAA ACAGGTTATA   | 5040 |
| ACAGTGTAA AAGTCTCAGT TTCTGCTTG GGGAACTTGT GTCCCTAATG TGTTAGATTG    | 5100 |
| CTAGATTGCT AAGGAGCTGA TACTTGACAG TTTTTAGAC CTGTGTTACT AAAAAAAAGA   | 5160 |

|   |      |
|---|------|
| TGAATGTCGG AAAAGGGTGT TGGGAGGGTG GTCAACAAAG AAACAAAGAT GTTATGGTGT | 5220 |
| TTAGACTTAT GGTTGTTAAA AATGTCATCT CAAGTCAGT CACTGGTCTG TTTGCATTTG  | 5280 |
| ATACATTTT GTACTAACTA GCATTGTAAA ATTATTCAT GATTAGAAAT TACCTGTGGA   | 5340 |
| TATTTGTATA AAAGTGTGAA ATAAATTTT TATAAAAGTG TTCATTGTTT CGTAACACAG  | 5400 |
| CATTGTATAT GTGAAGCAAA CTCTAAAATT ATAAATGACA ACCTGAATTA TCTATTCAT  | 5460 |
| CAAAAAAAA AAAAAAAA ACTTTATGGG CACAACTGG                           | 5499 |

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 141 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: internal
- (ix) FEATURE:
  - (A) NAME/KEY: Region
  - (B) LOCATION: 1..141
  - (D) OTHER INFORMATION: /note= "The 141 amino acid sequence predicted from the nucleic acid product which results from amplification of the mouse ALPHA 6B cDNA with primers 1157/1156."
- (ix) FEATURE:
  - (A) NAME/KEY: Domain
  - (B) LOCATION: 88..113
  - (D) OTHER INFORMATION: /note= "The putative transmembrane domain."
- (ix) FEATURE:
  - (A) NAME/KEY: Region
  - (B) LOCATION: 1..120
  - (D) OTHER INFORMATION: /note= "SEQ ID NO:5 is identical to SEQ ID NO:7 at amino acid position 1 through 120; the two sequences diverge at amino acid 121."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Thr Leu Asn Cys Ser Val Asn Val Arg Cys Val Asn Ile Arg Cys Pro  
1 5 10 15

Leu Arg Gly Leu Asp Ser Lys Ala Ser Leu Val Leu Arg Ser Arg Leu  
20 25 30

Trp Asn Ser Thr Phe Leu Glu Glu Tyr Ser Lys Leu Asn Tyr Leu Asp  
35 40 45

Ile Leu Leu Arg Ala Ser Ile Asp Val Thr Ala Ala Ala Gln Asn Ile  
50 55 60

Lys Leu Leu Thr Ala Gly Thr Gln Val Arg Val Thr Val Phe Pro Ser  
65 70 75 80

Lys Thr Val Ala Gln Tyr Ser Gly Val Ala Trp Trp Ile Ile Leu Leu  
85 90 95

Ala Val Leu Ala Gly Ile Leu Met Leu Ala Leu Leu Val Phe Leu Leu  
100 105 110

Trp Lys Cys Gly Phe Phe Lys Arg Ser Arg Tyr Asp Asp Ser Ile Pro  
115 120 125

Arg Tyr His Ala Val Arg Ile Arg Lys Glu Glu Arg Glu  
130 135 140

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 426 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1..426
- (D) OTHER INFORMATION: /product= "Mouse ALPHA 6B amino acid sequence in SEQ ID NO:5."

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature

(B) LOCATION: 262..337  
 (D) OTHER INFORMATION: /function= "Putative transmembrane region."

## (ix) FEATURE:

(A) NAME/KEY: misc\_feature  
 (B) LOCATION: (342<sup>343</sup>)  
 (D) OTHER INFORMATION: /note= "SEQ ID NO:6 is identical to SEQ ID NO:8 except for 130 nucleotides present in SEQ ID NO:8 but deleted between nucleotides 342 and 343 of SEQ ID NO:6."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

|   |     |
|---|-----|
| GAECTTTAAC TGTAGCGTGA ACGTGAGGTG TGTGAACATC AGGTGCCAC TGCGAGGGCT    | 60  |
| GGACAGCAAG GCCTCTCTCG TTCTTCGTTC CAGGTTGTGG AACAGCACAT TTCTAGAGGA   | 120 |
| ATATTCCAAA CTGAACTACT TGGACATTCT CCTGAGGGCT TCCATAGATG TCACCGCTGC   | 180 |
| TGCTCAGAAT ATCAAGCTCC TCACCGCCGG CACTCAGGTT CGAGTGACGG TGTTCCCTC    | 240 |
| AAAGACTGTA GCTCAGTATT CAGGACTAGC TTGGTGGATC ATCCTCCTGG CTGTTCTTGC   | 300 |
| CGGGATTCTG ATGCTGGCTC TATTAGTGTGTT TTTACTGTGG AAGTGTGGAT TCTTTAAGCG | 360 |
| CTCTAGGTAC GATGACAGCA TTCCCCGATA CCATGCGGTG CGGATCCGGA AAGAAGAGCG   | 420 |
| AGAGAT  | 426 |

## (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 149 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

## (ix) FEATURE:

(A) NAME/KEY: Region  
 (B) LOCATION: 1..149  
 (D) OTHER INFORMATION: /note= "The 149 amino acid sequence predicted from the product which results from

90

amplification of the mouse ALPHA 6A cDNA with primers 1157/1156."

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..120
- (D) OTHER INFORMATION: /note= "SEQ ID NO:7 is identical to SEQ ID NO:5 at amino acid positions 1 through 120; the sequences diverge at amino acid 121."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

|     |     |     |     |     |     |     |     |     |     |     |     |      |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|
| Thr | Leu | Asn | Cys | Ser | Val | Asn | Val | Arg | Cys | Val | Asn | Ile  | Arg | Cys | Pro |
| 1   |     |     |     |     | 5   |     |     |     |     | 10  |     |      |     |     | 15  |
| Leu | Arg | Gly | Leu | Asp | Ser | Lys | Ala | Ser | Leu | Val | Leu | Arg  | Ser | Arg | Leu |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |      | 30  |     |     |
| Trp | Asn | Ser | Thr | Phe | Leu | Glu | Glu | Tyr | Ser | Lys | Leu | Asn  | Tyr | Leu | Asp |
|     |     |     |     | 35  |     |     |     | 40  |     |     |     |      | 45  |     |     |
| Ile | Leu | Leu | Arg | Ala | Ser | Ile | Asp | Val | Thr | Ala | Ala | Ala  | Gln | Asn | Ile |
|     |     |     |     |     | 50  |     |     | 55  |     |     |     | 60   |     |     |     |
| Lys | Leu | Leu | Thr | Ala | Gly | Thr | Gln | Val | Arg | Val | Thr | Val  | Phe | Pro | Ser |
|     |     |     |     | 65  |     |     | 70  |     |     | 75  |     |      | 80  |     |     |
| Lys | Thr | Val | Ala | Gln | Tyr | Ser | Gly | Val | Ala | Trp | Trp | Ile  | Ile | Leu | Leu |
|     |     |     |     | 85  |     |     |     | 90  |     |     |     | 95   |     |     |     |
| Ala | Val | Leu | Ala | Gly | Ile | Leu | Met | Leu | Ala | Leu | Leu | Val  | Phe | Leu | Leu |
|     |     |     |     |     | 100 |     |     | 105 |     |     |     | 110  |     |     |     |
| Trp | Lys | Cys | Gly | Phe | Phe | Lys | Arg | Asn | Lys | Lys | Asp | His  | Tyr | Asp | Ala |
|     |     |     |     | 115 |     |     |     | 120 |     |     |     | 125  |     |     |     |
| Thr | Tyr | His | Lys | Ala | Glu | Ile | His | Thr | Gln | Pro | Ser | Asp  | Lys | Glu | Arg |
|     |     |     |     |     | 130 |     |     | 135 |     |     |     | 140. |     |     |     |
| Leu | Thr | Ser | Asp | Ala |     |     |     |     |     |     |     |      |     |     |     |
|     |     |     |     | 145 |     |     |     |     |     |     |     |      |     |     |     |

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 556 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: misc\_feature  
(B) LOCATION: 1..556  
(D) OTHER INFORMATION: /product= "Mouse ALPHA 6A amino acid sequence in SEQ ID NO:7."  
/note= "SEQ ID NO:8 is the 556 base nucleotide sequence corresponding to the mouse ALPHA 6A amino acid sequence SEQ ID NO:7, plus the first 109 nucleotides in the 3' noncoding region."

(ix) FEATURE:

(A) NAME/KEY: misc\_feature  
(B) LOCATION: 342..472  
(D) OTHER INFORMATION: /note= "SEQ ID NO:8 is identical to  
SEQ ID NO:6 except it has a 130 base insertion  
(nucleotides 342-472 of SEQ ID NO:8) between  
nucleotides 352 and 353 of SEQ ID NO:6."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 153 amino acids
- (B) TYPE: amino acid

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 1..153

(D) OTHER INFORMATION: /note= "SEQ ID NO:9 is the 153  
amino acid sequence predicted from the product  
which results from amplification of the mouse  
ALPHA 3B cDNA with primers 2032/2033."

(ix) FEATURE:

(A) NAME/KEY: Domain

(B) LOCATION: 108..112

(D) OTHER INFORMATION: /note= "The cytoplasmic sequence  
CDFFK begins at amino acid position 108."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ala Arg Cys Val Trp Leu Glu Cys Pro Leu Pro Asp Thr Ser Asn Ile  
1 5 10 15

Thr Asn Val Thr Val Lys Ala Arg Val Trp Asn Ser Thr Phe Ile Glu  
20 25 30

Asp Tyr Lys Asp Phe Asp Arg Val Arg Val Asp Gly Trp Ala Thr Leu  
35 40 45

Phe Leu Arg Thr Ser Ile Pro Thr Ile Asn Met Glu Asn Lys Thr Thr  
50 55 60

Cys Phe Ser Val Asn Ile Asp Ser Lys Leu Leu Glu Glu Leu Pro Ala  
65 70 75 80

Glu Ile Glu Leu Trp Leu Val Leu Val Ala Val Gly Ala Gly Leu Leu  
85 90 95

Leu Leu Gly Leu Ile Ile Leu Leu Trp Lys Cys Asp Phe Phe Lys  
100 105 110

Pro Thr Arg Tyr Tyr Arg Ile Met Pro Lys Tyr His Ala Val Arg Ile  
115 120 125

93

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Glu | Glu | Asp | Arg | Tyr | Pro | Pro | Pro | Gly | Ser | Thr | Leu | Pro | Thr | Lys |
| 130 |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 140 |

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | His | Trp | Val | Thr | Ser | Trp | Gln | Ile |
| 145 |     |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     | 150 |

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 463 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1..463
- (D) OTHER INFORMATION: /product= "Mouse ALPHA 3B amino acid sequence in SEQ ID NO:9."  
/note= "SEQ ID NO:10 is the 463 base nucleotide sequence corresponding to the mouse ALPHA 3B amino acid sequence in SEQ ID NO:9."

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 324..338
- (D) OTHER INFORMATION: /product= "The cytoplasmic sequence CDFFK."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

|             |            |             |             |            |            |     |
|-------------|------------|-------------|-------------|------------|------------|-----|
| GTGCCCGCTG  | TGTGTGGCTG | GAGTGGCCCC  | TTCCAGACAC  | CTCCAACATT | ACCAATGTGA | 60  |
| CCGTGAAAGC  | ACGGGTGTGG | AACAGCACCT  | TCATTGAGGA  | CTACAAAGAC | TTTGACAGAG | 120 |
| TCAGGGTAGA  | TGGCTGGCT  | ACCCGTGTTCC | TGAGAACCAAG | CATCCCTACC | ATCAACATGG | 180 |
| AGAACAAAGAC | CACATGTTTC | TCTGTGAACA  | TTGACTCAAA  | GCTGTTGGAG | GAGCTGCCG  | 240 |
| CTGAGATTGA  | GCTGTGGTTG | GTGCTTGTGG  | CCGTGGCTGC  | TGGGTTGCTG | CTGCTGGGGC | 300 |
| TCATCATCAT  | CCTCTTGTGG | AAGTGTGACT  | TCTTTAAGCC  | GACCCGCTAC | TACCGGATTA | 360 |
| TGCCCAAGTA  | CCATGCAGTG | CGTATCCGGG  | AGGAGGACCG  | CTACCCACCT | CCAGGGAGCA | 420 |

CGCTACCCAC CAAGAAGCAC TGGGTACCCA GCTGGCAGAT TCG

463

## (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1..20
- (D) OTHER INFORMATION: /standard\_name= "PCR PRIMER 1157"  
/note= "Primer corresponds to bp 2918-2937 of the  
ALPHA 6A cDNA sequence of SEQ ID NO:2."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GACTCTAAC TGTAGCGTGA

20

## (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1..20
- (D) OTHER INFORMATION: /standard\_name= "PCR PRIMER 1156"  
/note= "The primer corresponds to the complement  
of bp 3454-3473 of the AFHA 6A cDNA sequence of  
SEQ ID NO:2."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATCTCTCGCT CTTCTTTCCG

20

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1..19
- (D) OTHER INFORMATION: /standard\_name= "PCR PRIMER 1681"  
/note= "The primer corresponds to bp 2942-2960 of  
the ALPHA 6A cDNA sequence of SEQ ID NO:2."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GAACTGTGTG AACATCAGA

19

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1..20
- (D) OTHER INFORMATION: /standard\_name = "PCR PRIMER 2002"

/note= "The primer corresponds to the complement of bp 3433-3452 of the ALPHA 6A cDNA sequence of SEQ ID NO:2."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATCCTTACAG CATGGTATCG

20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1..20
- (D) OTHER INFORMATION: /standard\_name= "PCR PRIMER 2032"  
/note= "The primer corresponds to the hamster ALPHA 3A cDNA sequence of Tsuji et. al., J. Biol. Chem., 265:7016-7021 (1990)."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AAGCCAAATC TGAGACTGTG

20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

97

## (ix) FEATURE:

(A) NAME/KEY: misc\_feature  
(B) LOCATION: 1..20  
(D) OTHER INFORMATION: /standard\_name= "PCR PRIMER 2033"  
/note= "The primer corresponds to the hamster  
ALPHA 3A cDNA sequence of Tsuji et al.. J. Biol.  
Chem., 265:7016-7021 (1990)."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GTAGTATCGG TCCCGAATCT

20

## What Is Claimed Is:

1. A polypeptide of about 24 to about 1091 amino acid residues in length having a sequence that includes the  $\alpha_{6B}$  cytoplasmic domain sequence shown in SEQ ID NO 3 from residue 1068 to residue 1091.  
5
2. The polypeptide of claim 1 having a sequence shown in SEQ ID NO 3 from residue 1068 to residue 1091.
3. The polypeptide of claim 1 having a sequence shown in SEQ ID NO 3 from residue 1045 to residue 1091.  
10
4. A polypeptide having an amino acid residue sequence shown in SEQ ID NO 3 from residue 1 to residue 1091.
5. A polypeptide of about 21 to about 141 amino acid residues in length having a sequence that includes the  $\alpha_{6B}$  cytoplasmic domain sequence shown in SEQ ID NO 5 from residue 121 to residue 141.  
15
6. The polypeptide of claim 5 having a sequence shown in SEQ ID NO 5 from residue 121 to residue 141.  
20
7. A polypeptide of about 41 to about 153 amino acid residues in length having a sequence that includes the  $\alpha_{3B}$  cytoplasmic domain sequence shown in SEQ ID NO 9 from residue 113 to residue 153.  
25
8. The polypeptide of claim 7 having a sequence shown in SEQ ID NO 9 from residue 113 to residue 153.
9. An antibody molecule that immunoreacts with the  $\alpha_{6B}$  protein and with a polypeptide having an amino acid residue sequence shown in SEQ ID NO 3 from residue 1068-1091.  
30
10. The antibody molecule of claim 9 wherein said antibody molecule is a monoclonal antibody molecule.  
35
11. An antibody molecule that immunoreacts with the  $\alpha_{6B}$  protein and with a polypeptide having an amino

acid residue sequence shown in SEQ ID NO 3 from residue 1045 to residue 1091.

12. The antibody molecule of claim 11 wherein said antibody molecule is a monoclonal antibody molecule.

5 13. An antibody molecule that immunoreacts with the  $\alpha_{6B}$  protein and with a polypeptide having an amino acid residue sequence shown in SEQ ID NO 5 from residue 121 to residue 141.

10 14. The antibody molecule of claim 13 wherein said antibody molecule is a monoclonal antibody molecule.

15 15. An antibody molecule that immunoreacts with the  $\alpha_{3B}$  protein and with a polypeptide having an amino acid residue sequence shown in SEQ ID NO 9 from residue 113 to residue 153.

16. The antibody molecule of claim 15 wherein said antibody molecule is a monoclonal antibody molecule.

20 17. A method for detecting the presence of antigen having the cytoplasmic domain of  $\alpha_{6B}$  in a body sample comprising the steps of:

25 a) admixing the body sample with a composition containing antibody molecules that immunoreact with the  $\alpha_{6B}$  protein and with a polypeptide consisting essentially of an amino acid residue sequence shown in SEQ ID NO 3 from residue 1045 to residue 1091 to form an immunoreaction admixture;

30 b) maintaining said immunoreaction admixture under immunoreaction conditions for a time period sufficient for said antibody molecules to immunoreact with any  $\alpha_{6B}$  present in said body sample and form an immunoreaction complex; and

35 c) detecting the presence of any immunoreaction complex formed in step (b) and thereby

detecting the presence of said antigen in said body sample.

18. The method of claim 17 wherein said detecting in step (c) comprises the steps of:

5 (i) admixing said immunoreaction product formed in step (b) with an indicating means to form a second reaction admixture;

10 (ii) maintaining said second reaction admixture for a time period sufficient for said indicating means to bind to the immunoreaction product formed in step (b) and form a second reaction product; and

15 (iii) determining the presence of said indicating means in said second reaction product, and thereby the presence of said immunoreaction product formed in step (b).

19. The method of claim 18 wherein said indicating means is a labeled antibody comprising an antibody having a label affixed thereto.

20 20. The method of claim 17 wherein said sample is a fluid sample, said admixing in step (a) includes admixing said body fluid sample and said antibody composition with a solid support comprising a solid matrix having affixed thereto a polypeptide having an amino acid residue sequence that includes an amino acid residue sequence shown in SEQ ID NO 3 from residue 1068 to residue 1091 such that said immunoreaction admixture is a competition immunoreaction admixture having a liquid phase and a solid phase, and said immunoreaction product formed in step (c) is in the solid phase.

30 21. The method of claim 20 wherein said antibody is a labeled antibody, having a label affixed to the antibody.

35 22. The method of claim 21 wherein said detecting in step (c) comprises determining the

presence of said label in the solid phase immunoreaction product, and thereby the presence of said immunoreaction product.

23. The method of claim 120 wherein said polypeptide has an amino acid residue sequence shown in SEQ ID NO 3 from residue 1045 to residue 1091.

24. The method of claim 17 wherein said sample is a fluid sample and said antibody molecules are affixed to a solid support such that said immunoreaction admixture is a competition immunoreaction admixture having a liquid phase and a solid phase, and said immunoreaction product formed in step (c) is in the solid phase.

25. The method of claim 24 wherein said admixing in step (b) includes admixing said body fluid sample and said solid-phase antibody composition with a polypeptide having an amino acid residue sequence that includes an amino acid residue sequence shown in SEQ ID NO 3 from residue 1068 to residue 1091 such that said immunoreaction admixture is a competition immunoreaction admixture.

26. The method of claim 25 wherein said polypeptide is a labeled polypeptide, having a label affixed to the polypeptide.

27. A method for detecting the presence of antigen having the cytoplasmic domain of  $\alpha_{6B}$  in a body sample comprising the steps of:

30 a) admixing the body sample with a composition containing antibody molecules that immunoreact with the  $\alpha_{6B}$  protein and with a polypeptide having an amino acid residue sequence shown in SEQ ID NO 5 from residue 121 to residue 141 to form an immunoreaction admixture;

35 b) maintaining said immunoreaction admixture under immunoreaction conditions for a time period sufficient for said antibody molecules to

immunoreact with any  $\alpha_{6B}$  present in said body sample and form an immunoreaction complex; and

5 c) detecting the presence of any immunoreaction complex formed in step (b) and thereby detecting the presence of said antigen in said body sample.

28. A method for detecting the presence of antigen having the cytoplasmic domain of  $\alpha_{3B}$  in a body sample comprising the steps of:

10 a) admixing the body sample with a composition containing antibody molecules that immunoreact with the  $\alpha_{3B}$  protein and a polypeptide having an amino acid residue sequence shown in SEQ ID NO 9 from residue 113 to residue 153 to form an 15 immunoreaction admixture;

b) maintaining said immunoreaction admixture under immunoreaction conditions for a time period sufficient for said antibody molecules to immunoreact with any  $\alpha_{3B}$  present in said body sample 20 and form an immunoreaction complex; and

c) detecting the presence of any immunoreaction complex formed in step (b) and thereby detect the presence of said antigen in said body sample.

25 29. A diagnostic system in kit form for assaying for the presence of  $\alpha_{6B}$  subunit in a body sample, comprising a package containing, in an amount sufficient to perform at least one assay, an antibody composition comprising antibody molecules that immunoreact with the  $\alpha_{6B}$  protein and with a 30 polypeptide having an amino acid residue sequence shown in SEQ ID NO 3 from residue 1045 to residue 1091.

35 30. The diagnostic system of claim 29 wherein said antibody is affixed to a solid matrix.

31. The diagnostic system of claim 29 that further includes a solid support comprised of a solid matrix having affixed thereto a polypeptide having an amino acid residue sequence that includes an amino acid residue sequence shown in SEQ ID NO 3 from residue 1068 to residue 1091.

5 32. The diagnostic system of claim 31 that further includes, in a separate package, labeled specific binding agent for signaling the presence of 10 an immunoreaction product in the solid phase.

15 33. A diagnostic system in kit form for assaying for the presence of  $\alpha_{6B}$  subunit in a body sample, comprising a package containing, in an amount sufficient to perform at least one assay, an antibody composition comprising antibody molecules that immunoreact with the  $\alpha_{6B}$  protein and with a polypeptide having an amino acid residue sequence shown in SEQ ID NO 5 from residue 121 to residue 141.

20 34. A diagnostic system in kit form for assaying for the presence of  $\alpha_{3B}$  subunit in a body sample, comprising separate packages containing, in an amount sufficient to perform at least one assay, an antibody composition comprising antibody molecules that immunoreact with the  $\alpha_{3B}$  protein and with a 25 polypeptide having an amino acid residue sequence shown in SEQ ID NO 9 from residue 113 to residue 153.

35. The diagnostic system of claim 32 that further includes a solid support comprised of a solid matrix having said antibody molecules affixed thereto.

1/9

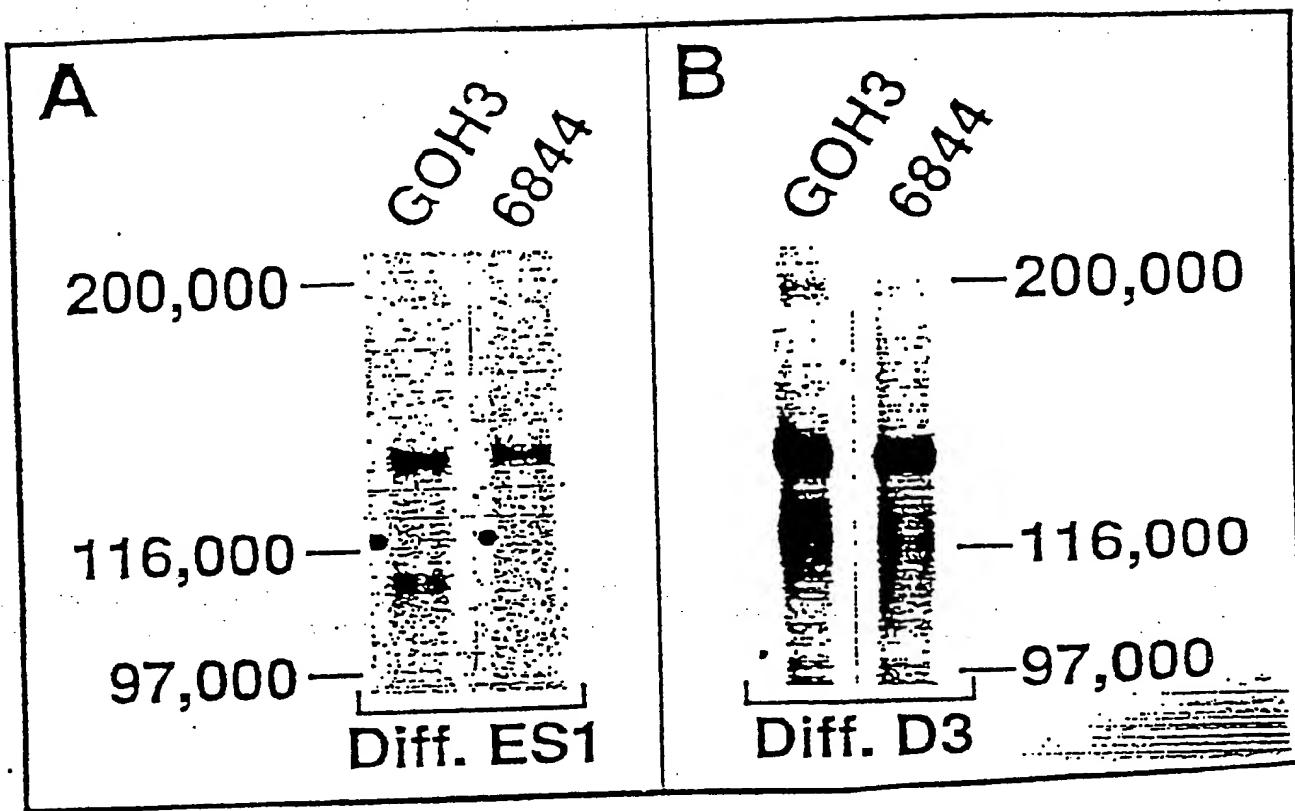


FIGURE 1

2/9

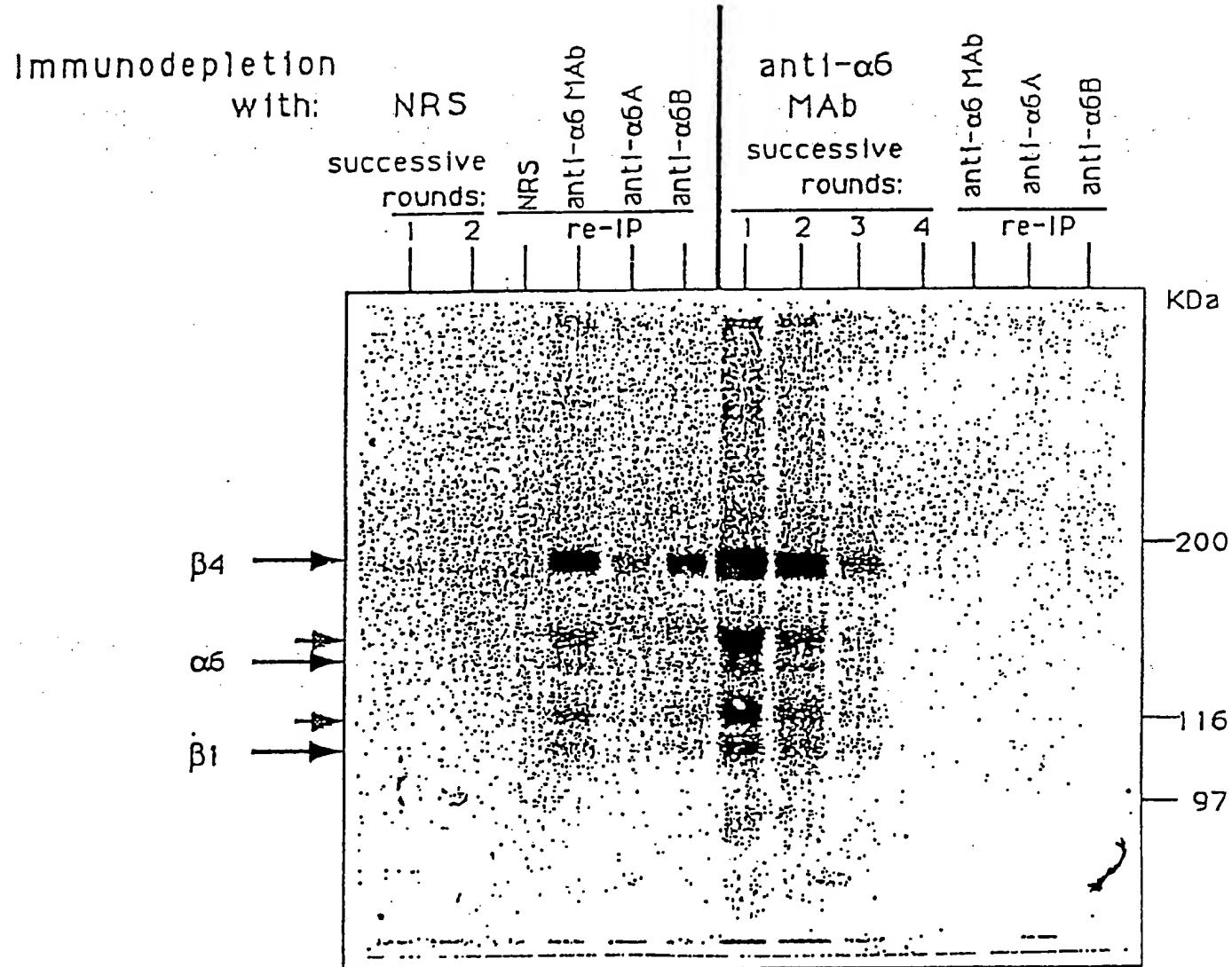


FIGURE 2

3/9

Immunodepletion  
with:

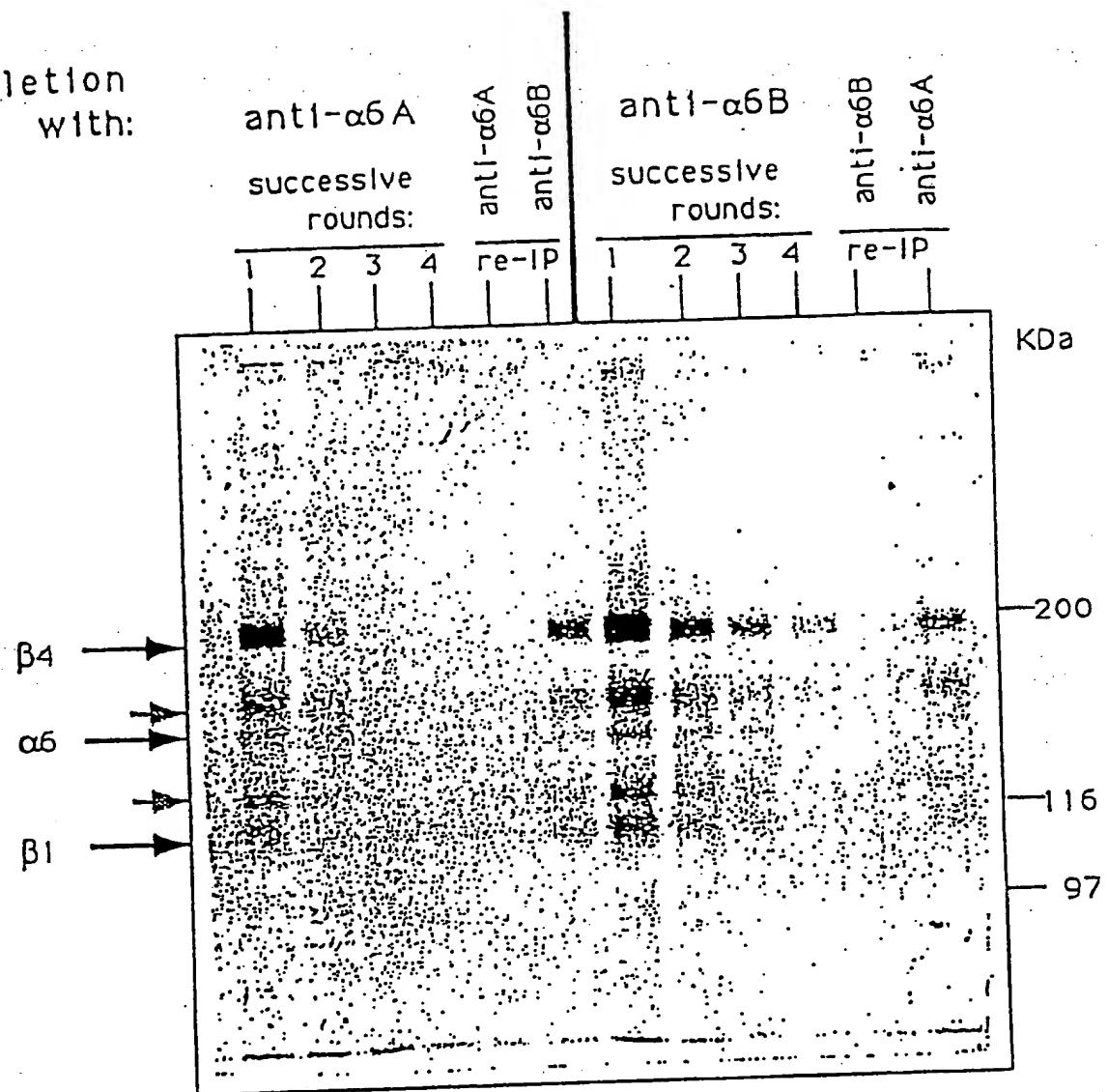


FIGURE 3

4/9

1 KB D06A FG JAR US37 1 KB

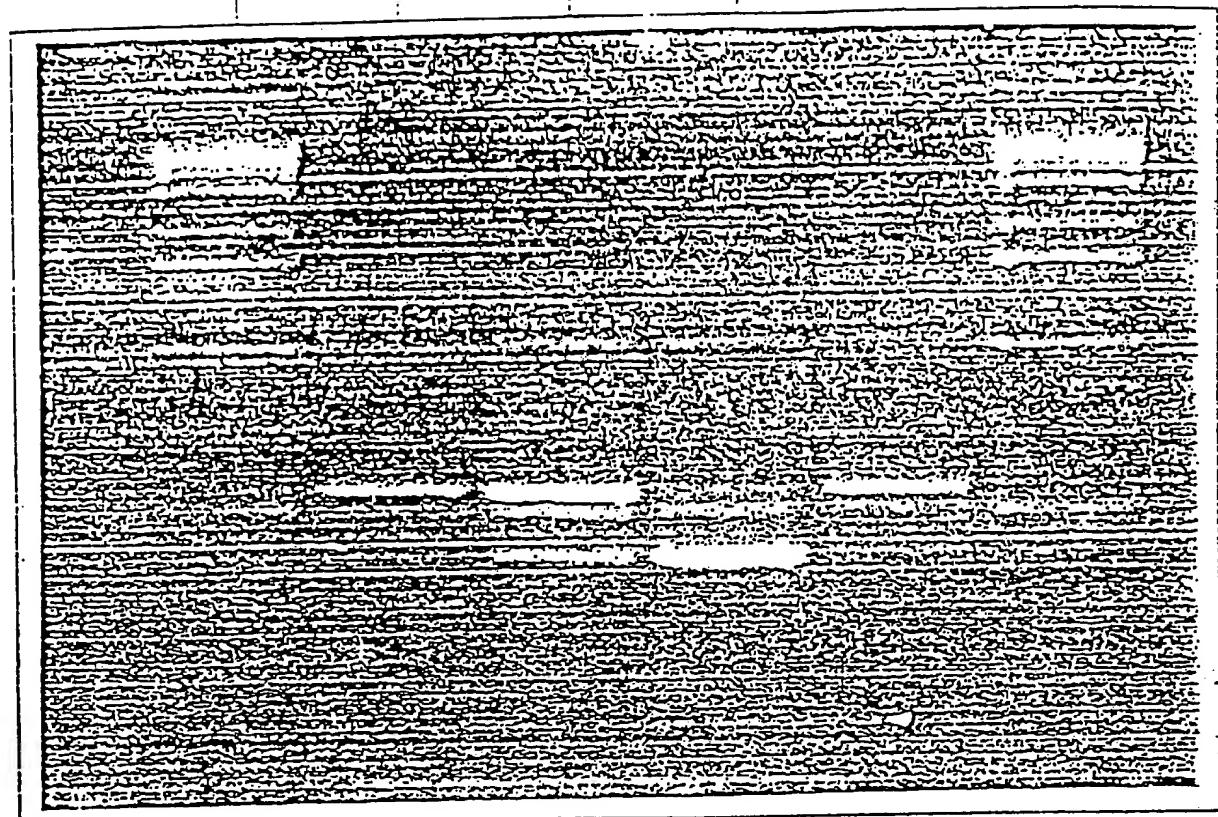


FIGURE 4

5/9

|             |      |  |      |
|-------------|------|--|------|
| $\alpha$ 6A | 2924 | TAACGTAGCGTGAACGTGAACCTGTGTGAACATCAGATGCCGCTGCAGGGGCTGGACAG  | 2963 |
|             | 1    |  | 60   |
| $\alpha$ 6B | 2984 | CAAGGCCTCTCTTATTTGCCTCGAGGTTATGGAACAGCACATTCTAGAGGATATT      | 3043 |
|             | 61   |  | 120  |
|             | 3044 | CAAACGTAACTACTGGACATTCTCATGCGAGCCTCATGATGTGACTGCTGCCGA       | 3103 |
|             | 121  |  | 180  |
|             | 3104 | AAATATCAGGCTGCCAATGCAGGCACTCAGGTTGAGTGAACGTGTTCCCTCAAAGAC    | 3163 |
|             | 181  |  | 240  |
|             | 3164 | TGTAGCTCAGTATTCGGGAGTACCTTGGTGGATCATCCTAGTGGCTATTCTCGCTGGGAT | 3223 |
|             | 241  |  | 300  |
|             | 3224 | CTTGATGCTTATTAGTGTAACTATGGAAGTGTGGTTCTCAAGAGAATAA            | 3283 |
|             | 301  |  | 360  |
|             | 3284 | CTTGATGCTTATTAGTGTAACTATGGAAGTGTGGTTCTCAAGAGAATAA            | 3343 |
|             | 361  | .....  | 420  |
|             | 3344 | AGAGAGGCTTACTTCTGATGCAAGTATTGATCTACTTCTGATTGTGGATTCTTA       | 3403 |
|             | 421  |  | 480  |
|             | 3404 | AACGCTCTAGGTACGATGACAGTGTCCCCGATACCATGCTGTAAGGATCCG          | 3455 |
|             | 461  |  | 532  |
|             |      | AACGCTCTAGGTACGATGACAGTGTCCCCGATACCATGCTGTAAGGATCCG          |      |

FIGURE 5

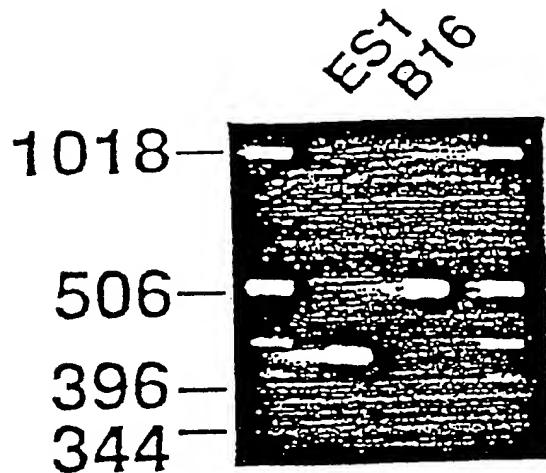
6/9

|      | 1157   | 1681 |      |
|------|--|------|------|
| 2951 | GCTGAAAGAAATAACCAAGACTCTTAACTCTAGCGTGAACGTGAACTGTGTGAACTCATCGA<br>A E R K Y O T L H C S V N V N C V N I R    |      | 2960 |
| 2961 | TGCCCCGCTGCCGGGGCTGGACACCCAGCGCTCTCTTATTTGCGCTCGAGGTTATGGAAC<br>C P L R G L D S K A S L I L R S R L W N      |      | 3020 |
| 3021 | AGCACATTTCTAGAGGAATATTCCAACTGAACTACTGGACATTCTCATGCCAGGCC<br>S T F L E E Y S K L N Y L D I L M R A F          |      | 3080 |
| 3081 | ATTGATGTGACTGCTGCTGCCAAAAATATCAGGCTGCCAAATGCAGGCACTCAGGTTCGA<br>I D V T A A A E N I R L P N A G T O V R      |      | 3140 |
| 3143 | GTGACTGTGTTCCCTAAAGACTGTAGCTCAGTATTGGGAGTACCTGGTGGATCATC<br>V T V F P S K T V A Q Y S G V P W H I            |      | 3200 |
| 3201 | CTAGTGGCTATTCTCGCTGGATCTGATGCTTATTAGTGTATACATGGAG<br>I V A I L A G I L M I A L I V F I L W K                 |      | 3260 |
| 3261 | TGTGGTTCTCAAGAGAAATAAGAAAGATCATTATGATGCCACATATCACAGGCTGAG<br>E G F F K R N K K D H Y D A T Y H K A E         |      | 3320 |
| 3321 | ATCCATGCTCAGCCATCTGATAAGAGAGGGTTACTTCTGATGCATAGTATTGATCTACT<br>I H A Q P S D K E R L T S D A * 2002          |      | 3380 |
| 3381 | TCTGTAATTGCTGGATTCTTAAACGCTCTAGGTACGTGACAGTGTCCCCGATAACCA<br>C G F F K R N K D H Y D D S V P R Y H           | 1150 | 3440 |
| 3441 | TGCTGTAAGGATCCGGAAAGAAGAGCGAGAGATCAAAGATGAAAGTATATTGATAACCT<br>A V R I R K E P R K E P K D E K K Y I D Y N L |      | 3500 |
| 3501 | TGAAAAAAACAGTGATCACAAAGTGGACAGAAATGAAAGCTACTCATAGCGGGGGCC<br>E K K Q W I T K W N R N E S Y S *               |      | 3560 |
| 3561 | TAAAAAAGCTTCACAGTACCCAACTGCTTTTC 3600  |      |      |

## FIGURE 6

7/9

A.



B.

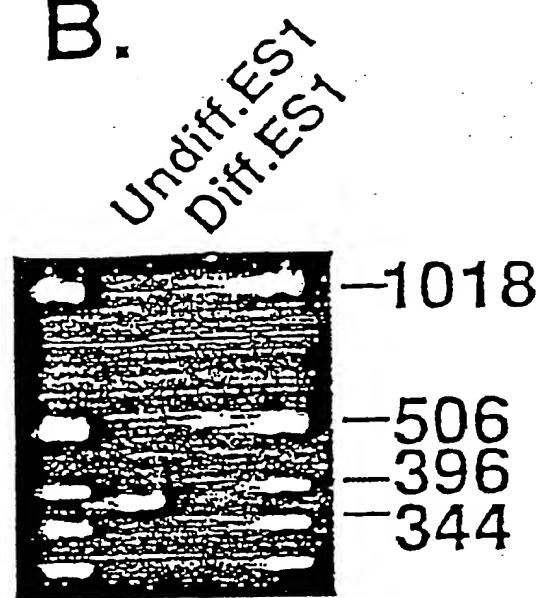


FIGURE 7

8/9

1157 →

gactcttaactgttagcgtaacgtgagggtgtgaacatcaggtgcccactgcgagggt 2976  
 gactcttaactgttagcgtaacgtgagggtgtgaacatcaggtgcccactgcgagggt  
 T L N C S V N V R C V N I R C P L R G L

ggacagcaaggcctctcggtttccaggttggaaacagcacattctagagga 3036  
 ggacagcaaggcctctcggtttccaggttggaaacagcacattctagagga  
 D S K A S L V L R S R L W N S T F L E E

atattccaaactgaactacttggacattctcggtttccatagatgtcaccgctgc 3096  
 atattccaaactgaactacttggacattctcggtttccatagatgtcaccgctgc  
 Y S K L N Y L D I L L R A S I D V T A A

tgctcagaatatcaagctcctcaccgcccggcactcaggttcgagtgacgggtttcc 3156  
 tgctcagaatatcaagctcctcaccgcccggcactcaggttcgagtgacgggtttcc  
 A Q N I K L L T A G T Q V R V T V F P S

aaagactgttagctcagttcaggagtagcttggatcatcctcctgggtttcc 3216  
 aaagactgttagctcagttcaggagtagcttggatcatcctcctgggtttcc  
 K T V A Q Y S G V A W W I I L L A V L A

cggttctgtatgtggcttatttagtgtttactgtggaa..... 3276  
 cggttctgtatgtggcttatttagtgtttactgtggaa.....  
 G I L M L A L L V F L L W K C G F F K R

..... 3336  
 aaataagaaaagatcattacgtgccacctatcacaaggctgagatccataactcagccgtc  
 N K K D H Y D A T Y H K A E I H T Q P S

.....gtgtggat 3396  
 tgataaagagaggctacttccgtacgtcatagtattgtatctacttccataattatataat  
 D K E R L T S D A \* C G F

tcttaaggcgtctaggtacgtacagcattccccgataccatgcggtgccggatccgg 3456  
 tcttaaggcgtctaggtacgtacagcattccccgataccatgcggtgccggatccgg  
 F K R S R Y D D S I P R Y H A V R I R K

1156

aagaagagcgagagat 3516

aagaagagcgagagat

E E R E

FIGURE 8

9/9



A



B

FIGURE 9

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/03527

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07K 7/10, 13/00, 15/28; G01N 33/68

US CL :530/324, 325, 326, 350, 387.9, 388.22; 435/7.21

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/324, 325, 326, 350, 387.9, 388.22; 435/7.21

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
chemical abstract service search terms: integrin, alpha subunit

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|-----------|---|-----------------------|
| X, P<br>Y | Eur. Journal of Biochemistry, Vol 199, issued July, 1991, R. Hogervorst et al., "Molecular cloning of the human $\alpha 6$ integrin subunit", pages 425-433. See abstract and Fig. 2.   | 1-4<br>9-12           |
| Y         | Journal of Biological Chemistry, Vol. 264, issued 15 April 1989, M. Hemler et al., "Association of the VLA $\alpha 6$ Subunit with a novel Protein", pages 6529-6535. See Fig. 5.   | 1-4, 9-12             |
| Y         | EMBO Journal, Vol. 8, no. 3, issued 1989 S. Kajiji et al., "A novel integrin ( $\alpha E \beta 4$ ) from human epithelial cells suggests a fourth family of integrin adhesion receptors", pages 673-680. See abstract.  | 7, 8, 15, 16, 28, 34  |
| Y         | Journal of Biological Chemistry, Vol. 265, no. 12, issued 25 April 1990, Tsuji et al. "Characterization through cDNA Cloning of Galactoprotein b3 (Gap b3), a Cell Surface Membrane Glycoprotein Showing Enhanced Expression on Oncogenic Transformation", pages 7016-7021. See abstract. | 7, 8, 15, 16, 28, 34  |

|                                     |  |                          |                          |
|-------------------------------------|--|--------------------------|--------------------------|
| <input checked="" type="checkbox"/> | Further documents are listed in the continuation of Box C. | <input type="checkbox"/> | See patent family annex. |
|-------------------------------------|--|--------------------------|--------------------------|

|   |     |  |
|---|-----|--|
| * Special categories of cited documents:  | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  |
| "A" document defining the general state of the art which is not considered to be part of particular relevance   |     |  |
| "E" earlier document published on or after the international filing date  | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone   |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "O" document referring to an oral disclosure, use, exhibition or other means  | "&" | document member of the same patent family  |
| "P" document published prior to the international filing date but later than the priority date claimed  |     |  |

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|---|---|
| Date of the actual completion of the international search<br>21 July 1992 | Date of mailing of the international search report<br>29 JUL 1992 |
|---|---|

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| Name and mailing address of the ISA/<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231 | Authorized officer<br>NINA OSSANNA, PHD |
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/03527

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.       |
|-----------|--|-----------------------------|
| X         | Journal of Biological Chemistry, vol. 263, no. 16, issued 05 June 1988, M. Hemler et al., "Multiple Very Late Antigen (VLA) Heterodimers on Platelets", pages 7660-7665, see page 7660 abstract and column 2, and Table I. | 1-6, 9-14, 17-27, 29-33, 35 |
| A         | Cell Differentiation and Development, Vol., 32, issued 1990, V. Quaranta, "Epithelial Integrins", pages 361-366, see entire document.  | 1-35                        |